CHROM. 4831

Gas–liquid chromatographic determination of dimethylnitrosamine as dimethylnitramine at picogram levels

In view of the extreme carcinogenicity of some of the nitrosamines in different animal species[1,2] and their reported occurrence in foodstuffs[3–6] considerable interest has developed, during the past few years, in chromatographic and other procedures for the detection of these compounds[7–13]. Polarographic[14], thin-layer (TLC) and gas–liquid chromatographic (GLC) methods have been used for their detection in foods with varying degree of success[7–13]. The polarographic method is only sensitive to 1 p.p.m. levels and it lacks specificity[12,13]. The TLC and GLC methods are more sensitive and specific than the polarographic technique but they require a large sample size (0.2–1 kg), and the clean-up steps are quite laborious and lengthy. These methods are unsuitable for analyzing small amounts of biological samples that are encountered during the metabolic studies of nitrosamines in laboratory animals. There is a need, therefore, for a more sensitive method of detecting these compounds.

An attempt to increase the sensitivity of the method by reducing the nitrosamines to hydrazines, prior to GLC, has proven unsuccessful[8]. The detection limit of the hydrazines was no better than that of the nitrosamines. During the course of our investigation we have observed that dimethylnitramine (DMNA), the oxidation product of dimethylnitrosamine (DMN), is extremely sensitive to electron capture detection, and this technique can be used to detect DMN at minute quantities. This communication reports the results of our findings.

Experimental

Reagents and solvents. All reagents were of analytical grade. Ethyl acetate, methylene chloride, *n*-pentane and *n*-hexane were distilled in all-glass apparatus.

Preparation of DMNA. Crystalline DMNA was prepared by nitrolysis of N,N-dimethylformamide according to the method of ROBSON[15]. The product was recrystallized from diethyl ether and *n*-pentane, and the final material had a m.p. of 52° (literature value, 54–56°). The IR spectrum (KBr pellet) showed peaks at 1470, 1437, 1385 and 1310 cm^{-1} indicating the presence of a nitro group. The compound was dissolved in ethyl acetate to give a concentration of 1 mg/ml and subsequent dilutions were carried out in *n*-pentane.

Conversion of DMN to DMNA. The method of EMMONS AND FERRIS[16] was used with minor modifications. About 1–5 μg of DMN in 2–10 μl of methylene chloride was added to 9 ml of trifluoroacetic acid and 50% hydrogen peroxide mixture (5:4) and the solution was allowed to stand at room temperature for 12–24 h. The mixture was poured on 10–15 g ice, made alkaline (pH 10–11) by careful addition of 30–40 ml 20% potassium carbonate, and extracted with two 50 ml portions of methylene chloride. The methylene chloride extract was dried over anhydrous sodium sulfate, filtered, and concentrated to *ca.* 5 ml by evaporating on a steam bath. The concentrated extract was quantitatively transferred into a glass-stoppered graduated test tube and 1 ml *n*-hexane was added. The solution was then concentrated to 0.2 ml on a hot water bath in a stream of nitrogen (care should be taken to avoid complete drying).

The solution was made up to 5–10 ml with *n*-pentane and a 1–4 μl aliquot was used for GLC analysis.

Isolation of DMN from nitrite-treated fish. The sample was prepared and extracted as described by us earlier[17]. The only difference was that all the concentration steps were carried out by evaporation through Snyder columns (macro and micro) instead of in a stream of nitrogen. The spot corresponding to DMN on the TLC plate was eluted with methylene chloride using a micro-Soxhlet apparatus. About 1 ml water was added to the extract and the mixture heated on a water bath (Snyder column) until all the methylene chloride was driven off. The aqueous solution was cooled to room temperature and the DMN present in the solution was converted to DMNA by the method described above. The preparation was passed through an alumina column (Woelm basic, cationotropic, activity grade 1; 2.5 cm × 1 cm diameter) and the interfering materials were removed by washing with 50 ml *n*-pentane. Finally, the adsorbed DMNA was eluted with 100 ml diethyl ether, the eluate concentrated to 0.5 ml, and a 0.2μl aliquot used for GLC analysis.

GLC analysis. A Varian Aerograph gas chromatograph, Model 1200, equipped with an electron capture detector (^{3}H) and a 1 mV recorder was used. Conditions:

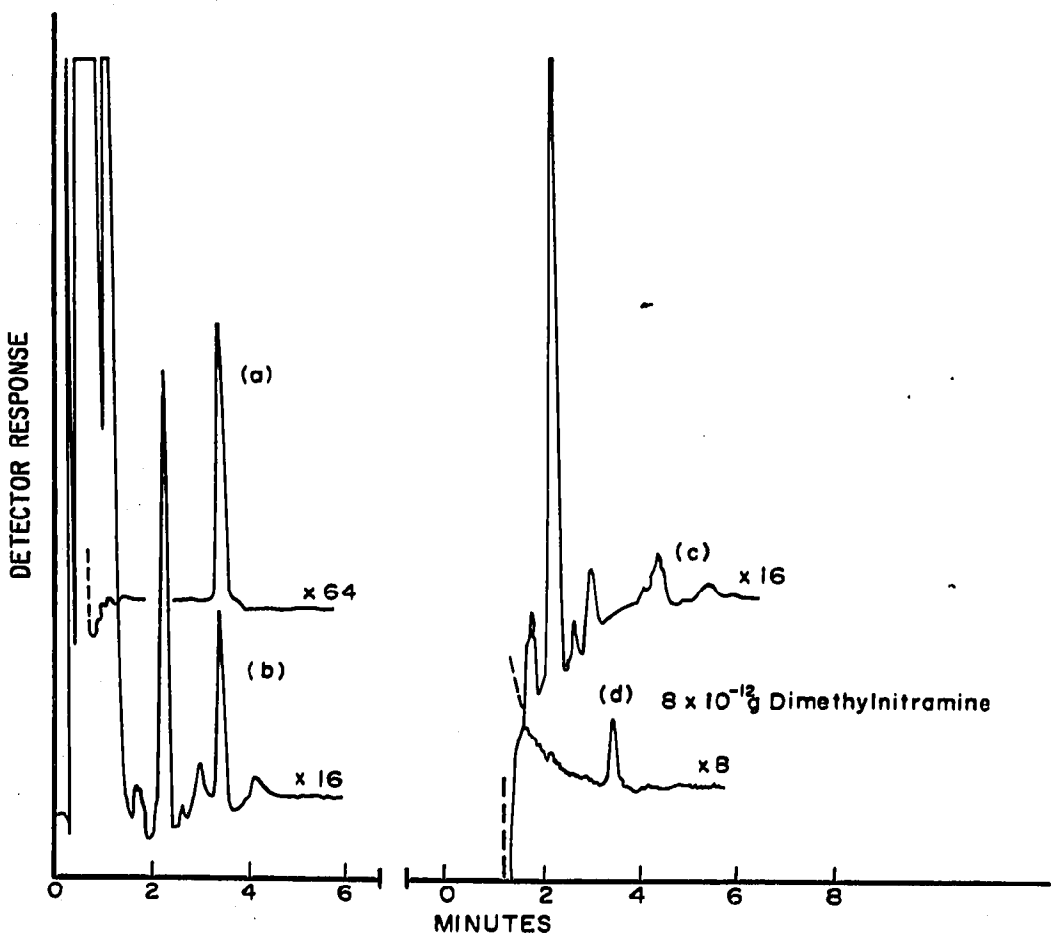

Fig. 1. GLC diagrams: (a) DMNA from nitrite-treated smoked hake (after clean-up through the alumina column), (b) DMNA prepared from DMN by pertrifluoroacetic acid oxidation, (c) pertrifluoroacetic acid oxidation blank and (d) DMNA prepared by nitrolysis of dimethylformamide. Range setting, 1; attenuator setting as shown in the diagrams. For other conditions see text.

10% Carbowax 20 M on 60–80 mesh chromosorb W (HMDS treated), 6 ft. × 1/8 in. stainless-steel column; column 152°, injector 225°, detector 210°, nitrogen flow 24 ml/min.

Results and discussion

The use of an electron capture detector made the GLC method very sensitive; about 8 pg of DMNA (or 16 pg DMN) could be detected under the conditions used (Fig. 1). This is about a thousand times more sensitive than the detection limit of nitrosamines by the hydrogen flame detector[12,17]. The peak heights were proportional to the amount of DMNA at least up to 100 pg (Fig. 2). A blank carried out through all the steps did not show the corresponding peak for DMNA (Fig. 1). The DMNA prepared by nitrolysis of dimethylformamide was undistinguishable from that prepared by pertrifluoroacetic acid oxidation of DMN.

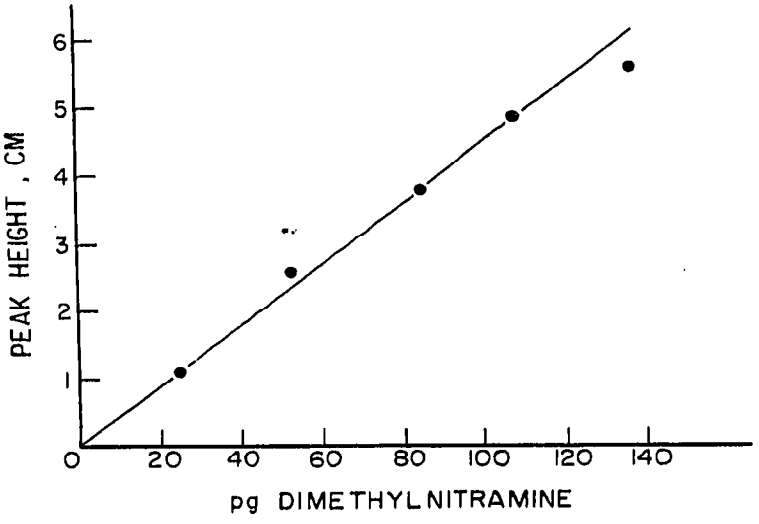

Fig. 2. Standard curve for DMNA.

The percentage conversion of DMN to DMNA varied with the conditions used but the results were reproducible. About 60% of the theoretical yield was obtained when the reaction mixture was allowed to stand for 24 h. An overnight reaction period at room temperature gave about 50% yield. The amount of DMNA formed from 5 μg DMN was proportional to that obtained from a 1 μg sample. The use of 90% hydrogen peroxide would be expected to produce a higher yield[16]. Due to relative unavailability of 90% hydrogen peroxide and the hazard involved in its use no attempt was made to use the concentrated reagent.

Thus far, we have shown that DMN can be oxidized to DMNA on a micro scale and detected by GLC at picogram levels. A great deal of work remains to be carried out before it can be directly applied to food extracts. Proper clean-up procedures need to be developed and applied to different foods. However, the technique, as it stands now, can be used to confirm the identity of nitrosamines isolated from foods by the existing procedures. To demonstrate this, we isolated DMN from a sample of nitrite-

treated smoked hake which was found to contain DMN in our previous studies[17]. A strong peak corresponding to DMNA (Fig. 1) thus confirms the identity of DMN.

Although we have only used the technique for the determination of DMN, it is anticipated that other volatile nitrosamines could be similarly converted to the nitramines and detected by GLC. It is hoped that the extreme sensitivity of the technique will be useful for studying the metabolism of various nitrosamines as well as for detecting their presence in the environment.

The author wishes to thank Mrs. BARBARA DONALDSON for technical assistance and Mr. R. C. O'BRIEN for taking the infrared spectrum.

Food and Drug Research Laboratories, N. P. SEN
Department of National Health and Welfare,
Ottawa, Ontario (Canada)

1 P. N. MAGEE AND J. M. BARNES, *Brit. J. Cancer*, 10 (1956) 114.
2 D. SCHMÄHL AND H. OSSWALD, *Experientia*, 23 (1967) 497.
3 J. SAKSHAUG, E. SÖGNEN, M. A. HANSEN AND N. KOPPANG, *Nature*, 206 (1965) 1261.
4 E. KRÖLLER, *Deut. Lebensm.-Rundschau*, 10 (1967) 303.
5 F. ENDER AND L. ČEH, *Food. Cosmet. Toxicol.*, 6 (1968) 569.
6 L. S. DU PLESSIS AND J. R. NUNN, *Nature*, 222 (1969) 1198.
7 R. PREUSSMANN, G. NEURATH, G. WULF-LORENTZEN, D. DAIBER AND H. HENGY, *Z. Anal. Chem.*, 202 (1964) 187.
8 W. J. SERFONTEIN AND P. HURTER, *Nature*, 209 (1966) 1238.
9 F. ENDER AND L. ČEH, *Alkylierend Wirkende Verbindungen*, Second Conference on Tobacco Research, Freiburg, 1967, p. 83.
10 H.-J. PETROWITZ, *Arzneimittel-Forsch.*, 18 (1968) 1486.
11 N. P. SEN, D. C. SMITH, L. SCHWINGHAMER AND J. J. MARLEAU, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 47.
12 J. K. FOREMAN, J. F. PALFRAMAN AND E. A. WALKER, *Nature*, 225 (1970) 554.
13 J. H. HOWARD, T. FAZIO AND J. O. WATTS, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 269.
14 O. G. DEVIK, *Acta Chem. Scand.*, 21 (1967) 2302.
15 J. H. ROBSON, *J. Am. Chem. Soc.*, 77 (1955) 107.
16 W. D. EMMONS AND A. F. FERRIS, *J. Am. Chem. Soc.*, 75 (1953) 4623.
17 N. P. SEN, D. C. SMITH, L. SCHWINGHAMER AND B. HOWSAM, *Can. Inst. Food. Technol. J.*, 3 (1970) 66.

Received May 4th, 1970

. Chromatog., 51 (1970) 301–304

:HROM. 4846

ieparation of carcinogenic nitrosamines on Sephadex LH-20

Chromatographic separations are well-established methods for the analysis of N-nitroso compounds. Thin-layer chromatography on silica gel or alumina[1] and a ariety of gas chromatographic methods have been successfully utilised for the sepaation and identification of these substances[2]. During our investigations of suitable ıethods for trace analysis of N-nitroso compounds[3–5] we examined whether gel hromatography could be employed as a separation technique for nitrosamines and ossibly also for the removal of interfering contaminants.