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GLC ANALYSIS OF N-NITROSODIMETHYLAMINE IN FISH PRODUCTS

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ABSTRACT

The contents of N-nitrosodimethylamine (DMNA) in fish products have been determined by gas-liquid chromatography. DMNA was isolated by steam distrillation, then extracted into dichloromethane and analysed on a 25 m OV-17 glass capillary column using flame ionisation and nitrogen/phosphorus detectors in parallell. Concentrations of DMNA found were 0.1–4 ppm (mg/kg) in fish meal and 0–4 ppb (microgram/kg) in smoked fish products.

INTRODUCTION

Nitrosamines belong to a class of compounds that have been shown to be carcinogenic in laboratory experiments. (MAGEE and BARNES, 1967). They are widely distributed in the environment, and have been found in low levels in various foods. (GOUGH et al., 1977; FAZIO et al., 1972; HAVERY and FAZIO, 1977).

Recent techniques applied to the analysis of nitrosamines include polarography (LYDERSEN and NAGY, 1967), gas liquid chromatography (SKAARE and DAHLE, 1975; HURST, 1976), and high pressure liquid chromatography (KLIMISCH and AMBROSIUS, 1976). A gas liquid chromatographic metod for the determination of N-nitrosodimethylamine (DMNA) in fish meal is in routine use in this country. (SKAARE and DAHLE, 1975). While the method serves its purpose well on the mg/kg-level, shortcomings become apparent when the method is applied to other types of sample containing DMNA at the microgram/kg-level. For these samples packed columns do not provide sufficient resolution in some instances and a pronounced solvent tail tends to impede the quantitative determination.

These problems have been overcome by removing the water from the sample, and by using glass capillary columns.

A 25 m OV-17 column provided sufficient resolution for the samples of fish products analysed in this laboratory.

EXPERIMENTAL

Fifty gram samples of various smoked fish products bought in local shops were homogenized and steam-distilled by the method given by N.T. CROSBY et al. (1972). Vacuum distillates from fish meal were provided by the Norwegian Herring Oil and Herring Meal Industry's Research Institute. Three ml of distillate was extracted twice with three ml portions of dichloromethane. Dipropylnitrosamine (DPNA) was added as an internal standard corresponding to 0.02 microgram/gram in the samples of smoked products, or 2 microgram/gram for fish meal samples. Approximately 2 ml of the dichloromethane was removed through a distillation column having an experimental plate number of seven. One ml of n-hexane was added to the concentrate, and distillation was continued to a final volume of approximately 0.2 ml. Analysis showed that the distillate did not contain DMNA. One microliter of the concentrate was injected by the splitless technique on a 25 m OV-17 column (Perkin-Elmer), i.d. 0.25 mm. GLC conditions were: instrument, Perkin-Elmer F 22, nitrogen carrier gas, inlet pressure 0.5 bar, injector and detector temperature 250°, temperature program (fish meal), 2 min. at 90°, then 4°/min to 140° (smoked products) 1°/min 70–75°, then 10°/min to 200°. Some samples were also analysed on a 100 m OV-101 column (Perkin-Elmer), i.d. 0.25 mm, isothermal at 120° with splitless injection and an inlet pressure of 2.9 bar.

The column effluent was split 1:1 to flame ionisation and nitrogen/phosphorous detectors, and detector signals were monitored on a two-pen recorder. Detector gas flows were: FID, hydrogen 21 ml/min., air 230 ml/min., PND, hydrogen 3,2 ml/min. air 82 ml/min., detector purge gas, nitrogen 23 ml/min for both detectors.

Quantitative analyses were performed on the 25 m OV-17 column by measuring the DMNA peak height relative to the DPNA peak height, and by electronic integration of peak areas for analyses on the 100 m OV-101 column.

RESULTS AND DISCUSSION

Calibration. Calibration was performed by analysing known mixtures of DPNA, and DMNA covering the concentration ranges found in the samples. The following regression lines were calculated:

a, for mg/kg calibration runs,
peak height DMNA/peak height DPNA = 1.036 × (Conc. DMNA/conc. DPNA) – 0.005 (1)
r = 0.9966

Concentration range DMNA 0.1–6.0 mg/kg, DPNA 2.0 mg/kg, 0.1 mg/kg DMNA in the sample corresponds to 0.2 ng passing through the detector.

b, for microgram/kg calibration runs,
peak height DMNA/peak height DPNA = 1.375 × (conc. DMNA/conc. DPNA) + 0.043 (2)
r = 0.9760
Concentration range DMNA 1–10 microgram/kg, DPNA 20 microgram/kg, 1 microgram/kg DMNA in the sample corresponds to 2.5 ng passing through the detector.

Reproducibility of the GLC analyses. Ten injections of the same sample of fish meal gave the following results on the OV-17 column: mean value 1.4 mg/kg, standard deviation 0.16 mg/kg. Ten analyses of a sample of smoked cod gave 2.5 microgram/kg with a standard deviation of 0.53 microgram/kg.

Accuracy of the extraction and GLC steps. Known amounts of DMNA was added to samples of fish meal distillate. The distillates were then extracted with dichloromethane, worked up and analysed (Table 1). It is seen that the analytical value is found in the range 75–110 per cent of the true value. Recovery of DMNA added to foodstuffs is generally below 100 per cent. (SKAARE and DAHLE, 1975; CROSBY et al., 1972). The analytical results presented here have not been corrected, and are therefore minimum values.

Inferences. It has been reported that pyrazines are found in various fish products, and may interfere with the determination of DMNA. (KADAR and DEVIK, 1970). Under the present conditions, 2,5-dimethylpyrazine is well separated from both the DMNA and DPNA peaks in the chromatogram. The need for high-resolution capillary columns is evident from the complexity of a typical chromatogram as given in figure 1. Without the selectivity provided by the nitrogen detector in parallell with the FID, it is virtually impossible to pick out the DMNA and DPNA peaks.

Detection limit. Our experience indicates that the sensitivity of the nitrogen/phosphorus detector is equivalent to the FID for the nitrosamines, and that it is possible to detect 1 microgram/kg of DMNA in actual samples using the 25 m OV-17 column. The 100 m OV-101 column provides a detection limit of 0.05 microgram/kg.

Comparison with other methods. Fish meal samples were also analysed by the method of J.U. SKAARE and H.K. DAHLE (1975). There was a fair agreement between the two methods (Table 2).

DMNA content of fish products. DMNA was identified by its retention time relative to DPNA on two different columns, i.e. 100 m OV-101 and 25 m OV-17, both of internal diameter 0.25 mm. The identity was verified by comparison with standard mixtures of DMNA and DPNA, and by analysing

samples which had been spiked by addition of known amounts of DMNA and DPNA.

Samples of fish meal similar to those reported in this study have been analysed by GLC-MS (SKAARE and DAHLE, 1975), and the identity of DMNA has been confirmed.

The 100 m column demonstrated the absence of DMNA for some of the smoked fish products. For samples in which the presence of DMNA could not be excluded, the concentration of DMNA found (Table 3) was in the range reported recently in a study, in which the identity of DMNA was confirmed by high-resolution GLC-MS. (GOUGH et al., 1977). Thus while confirmation of the identity of DMNA is lacking in the present study, the results seem reasonable.

The DMNA concentrations found in smoked fish products were low and similar to those reported in fresh fish (GOUGH et al., 1977).

Thus the smoking treatment of fish may not result in an increased concentration of DMNA.

Table 1. Recovery of DMNA (mg/kg) added to steam distillates from fish meal

Sample no	Original	Added	Found	Per cent found
1	0	1,0	1,1	110
2	0	2,0	1,5	75
3	0,1	4,0	3,1	76
4	0,3	5,8	6,0	98

Table 2. Comparison of analytical methods

Sample		Present method	Routine method (SKAARE and DAHLE, 1975)
Fish meal	1	0,2 mg/kg	<0,3 mg/kg
	2	0,1	<0,3
	3	0,2	<0,3
	4	0,3	0,3
	5	0,1	<0,3
	6	3	3
	7	4	3
	8	2	4
	9	0,7	0,4
	10	1	3
	11	2	3
	12	2	2

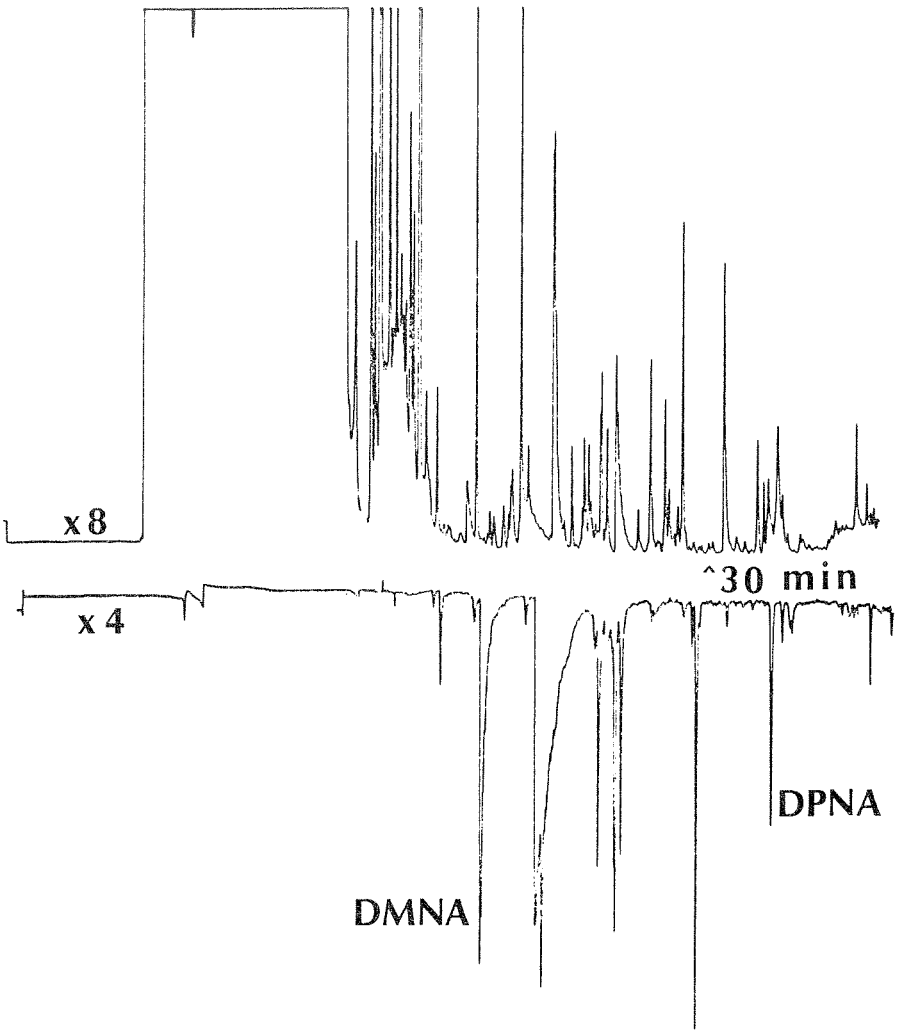

Figure 1.
Fish meal distillate
Glass WCOT Column
100 m x 0.25 mm OV–101
Upper trace flame ionisation detector
Lower trace Rb-silicate nitrogen detector

Table 3. DMNA in various fish products

Sample	microgram/kg
Sardines 1	2
» 2	2
» 3	4
» 4	<1
» 5	3
Smoked cod	3
» Greenland halibut	<0,05
» salmon	<0,05
» eel	1
Hot smoked mackerel	<0,05
Cold smoked mackerel	<0,05
Hot smoked small herring	2

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