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Note

Gas chromatographic determination and mass spectrometric confirmation of N-nitrosodimethylamine in fish meal

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N-Nitrosodimethylamine (DMNA) is a hepatotoxic compound and a potent carcinogen[1–4]. The presence of this compound in fish meal, made from fish preserved with nitrite, has long been known[5,6], and observations of the hepatotoxic disorders found in various domestic animals fed on fish meal containing DMNA have been published[5,7–11]. As a result of these effects, the fish meal produced in Norway during the last 7 years has been analyzed routinely for its DMNA content by procedures including colorimetry[12,13] and polarography[14]. Gas–liquid chromatography (GLC)[11] has also been tried. However, interference is caused in these methods by the pyrazines formed in fish meal by a Maillard reaction[14–16].

It appears from the literature that methods with sufficient specificity are limited to those in which mass spectrometry (MS) is used for the confirmation of the identity of the volatile DMNA, after quantitative determination by GLC[17,18]. However, most of the methods reported require extensive clean-up and solvent extraction techniques before DMNA analysis can be accomplished. Therefore, the need for a direct, rapid, sensitive and specific analytical method for the routine analysis of DMNA in fish meal resulted in the present study, which involved the direct GLC analysis and the MS confirmation of the DMNA content in the aqueous distillate obtained by vacuum distillation of fish meal containing 0.1–2.0 ppm of DMNA.

MATERIALS AND METHODS

Reagents

Standard N-nitrosamine reagents [N-nitrosodimethylamine (DMNA) and N-nitrosodipropylamine (DPNA)] for these analyses were Eastman Reagent Chemicals (Eastman-Kodak, Rochester, N.Y., U.S.A.), and 2,5-dimethylpyrazine (PYR) was obtained from K & K (Rare & Fine Chemicals; K & K Labs., Plainview, N.Y., U.S.A.). Versamid 900 was obtained from Supelco (Bellefonte, Pa., U.S.A.) and Chromosorb W from Applied Science Labs. (State College, Pa., U.S.A.). Dichloromethane and *n*-hexane were of analytical-reagent grade.

Procedure

DMNA was extracted from fish meal by vacuum distillation using the tech-

nique described by Lydersen and Nagy[14], slightly modified by the Norwegian Herring Oil and Herring Meal Industry's Research Institute. Samples of 20 and 40 g of fish meal were analyzed, and the volumes of the distillates were 50 and 25 ml, respectively. When 40 g of fish meal were analyzed, re-distillation was necessary in order to concentrate the distillate to 25 ml. DPNA was added to the distillate as the internal standard. Aliquots of 2–10 μl were examined by GLC using a nitrogen-selective detector and the quantitative determination was effected by comparison of peak areas using standard solutions of DMNA and the internal standard.

For the GLC–MS analysis of DMNA at the level of 0.1 ppm, the distillate had to be concentrated approximately 50-fold. Solvent extraction followed by concentration of the extract was carried out mainly according to Crosby *et al.*[19]; however, 25 ml of dichloromethane were used and the volume of the final concentrate was adjusted to 0.5–1.0 ml. Volumes of 2–10 μl of this extract were examined by GLC–MS.

Instrumental

Quantitative measurements of DMNA were carried out with a Varian Model 2100 gas chromatograph equipped with a nitrogen-specific detector (AFID) using a 2 m $\times$ 3 mm I.D. Pyrex column packed with 20% Versamid 900 (ref. 20) on 80–100 mesh Chromosorb W AW DMCS. The operating temperatures were as follows: column, programmed from 100 to 120° at 4°/min; injector, 150°; detector, 270°. Nitrogen at a flow-rate of 30 ml/min was used as the carrier gas and the flow-rates of hydrogen and air were adjusted for optimal conditions. The range and attenuator settings were 10^{-10}–10^{-12} and 32, respectively.

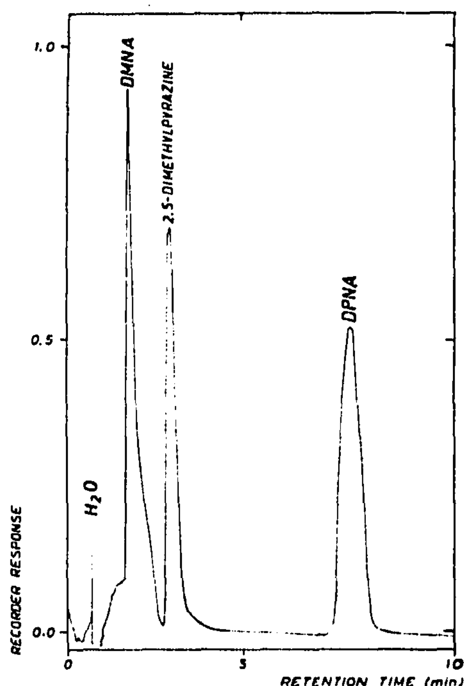
Analysis was also performed by using a Perkin-Elmer Model 900 gas chromatograph equipped with a nitrogen-specific detector.

For confirmation of the identity of the substances separated gas chromatographically, an LKB Model 900 mass spectrometer coupled to a gas chromatograph through a Becker-Ryhage separator was used. The column was as described above. The operating temperatures were as follows: column, 100°; flash heater, 150°; molecular separator, 200°; ion source, 250°. Helium at a flow-rate of 25 ml/min was used as the carrier gas. When recording gas chromatograms only, using the total ion current detector, the ion source was operated at 20 eV. When scanning mass spectra, the electron energy was changed to 70 eV automatically.

RESULTS AND DISCUSSION

The GLC system employed, with Versamid 900 as the liquid phase, readily separates DMNA, PYR and the internal standard (DPNA) when injected as an aqueous solution (Fig. 1). It can be seen that all of the substances are eluted within 10 min. By using a nitrogen-selective detector, DMNA at levels of 0.1–2.0 ppm in fish meal can be determined when aliquots of the distillate are injected directly. Examination of the eluted GLC peaks by MS showed that no interfering co-distillants are present.

Fig. 2 shows the gas chromatogram of 10 μl of the aqueous solution (25 ml) obtained from the vacuum distillation of 40 g of fish meal containing 1.1 ppm of DMNA. The mass spectrum of the DMNA isolated from the fish meal sample is



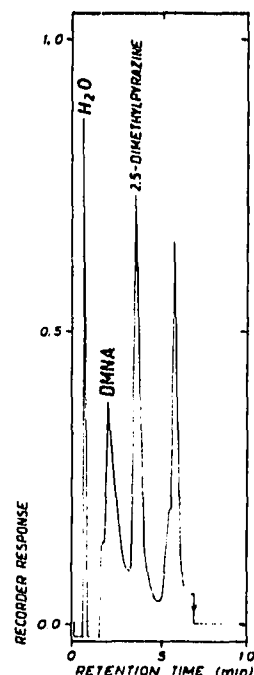


Fig. 1. Gas chromatogram of 3 μl of an aqueous solution containing 0.01 mg/ml each of DMNA, PYR and the internal standard DPNA, analyzed on a 20% Versamid 900 column, temperature programmed from 100 to 120° at 4°/min. An AFID was used and the attenuator and range settings were 32 and 10^{-12}, respectively.

Fig. 2. Gas chromatogram of 10 μl of the 25 ml of aqueous distillate prepared from 40 g of fish meal containing 1.1 ppm of DMNA. GLC parameters are as in Fig. 1.

identical with that of the standard and no interfering substances were found. The identity of the GLC peak eluted at 4 min is PYR, as confirmed by MS, while the identity of the GLC peak eluted at 6 min has not been examined. In this experiment, the internal standard was not added.

The quantitative determination of DMNA in fish meal was carried out by using the internal standard technique, adding DPNA to the distillate as the internal standard, and employing a nitrogen-selective detector. The recoveries of DMNA added to the fish meal at levels of 0.1–2.0 ppm were about 75%. The reproducible limit of determination by an alkali flame ionization detector (AFID, Varian Aerograph) was found to be approximately 10 ng, which corresponded to a level of 1 ppm of DMNA in the fish meal. Linearity studies over the range 10–500 ng indicated that the detector response followed a rectilinear relationship. However, by using a different nitrogen-selective detector (Perkin-Elmer), the sensitivity was increased approximately 25-fold and DMNA at the level of 0.1 ppm could routinely be determined. Linearity of the response was observed in the concentration range studied.

As the fish meal may contain different volatile substances, depending on the raw fish material, the time of catching and the different technologies of production, interfering co-distillants may occasionally occur. Therefore, the identity of the substances separated by GLC should be confirmed occasionally by MS.

For the absolute confirmation of identity by MS, 80 ng of DMNA must be

present, and up to a 50-fold concentration of the distillate is therefore required. In the solvent extraction–concentration steps necessary, a 10–20% loss of DMNA is observed. The loss, however, is dependent on the volume of the final extract.

The results obtained indicate that the GLC method described is a rapid and reliable procedure for the routine determination of DMNA at levels of 0.1–2.0 ppm in fish meal. The method was recently accepted by a Governmental Advisory Group responsible for additives and contaminants in fish meal, and the method is now being tested by the Official Norwegian Control Laboratory in this field.

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