

Gas-Liquid Chromatographic Determination of N-Nitrosodimethylamine in Ham

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A method is described for the gas chromatographic determination of N-nitrosodimethylamine in ham. The use of a commercial rubidium sulfate alkali flame ionization detector for nitrogen-containing compounds permits a simple cleanup procedure and a rapid analysis. This method consists of digesting the ham sample with methanolic KOH, distillation from aqueous alkali, washing the distillate with hexane, acidification and extraction of the aqueous layer with methylene chloride, and washing the extracts with base prior to concentration. The sensitivity of the method is 25 ppb; the average recoveries at this level range from 73 to 100%. Ten hams including 4 varieties were analyzed and found to contain less than 1 ppb apparent N-nitrosodimethylamine.

Because of the increased concern for potential carcinogens of the nitrosamine type in the food supply, many methods for analysis of nitrosamines have been developed. These published methods involve time-consuming, extensive cleanup procedures and final determination by polarography or by thin layer and/or gas-liquid chromatographic techniques (1-12). Those based on GLC appear to be the most promising, especially for analysis in the ppb range (6).

With the advent of the alkali flame ionization detector (AFID), which gives a selective response to nitrogen- and phosphorus-containing compounds (13-15), it was felt that the characteristics of this detector could be used to reduce the response of potentially interfering components. Therefore a sample would not require a rigorous cleanup procedure for analysis of nitrogen-containing components. The long-lived rubidium sulfate AFID, described by Hartmann (14) as having the same sensitivity to nitrogen-containing compounds (2 nitrogen atoms per molecule) and 10,000 times less sensitivity to *n*-hydrocarbons than the standard flame ionization detector (FID), is now commercially available.

This laboratory has been involved in an effort to develop a rapid, accurate method for determining N-nitrosodimethylamine (DMNA) at the

ppb level by utilizing apparatus generally available in most laboratories. The procedure described in this paper can be applied to meat and food products and can be expanded to include other distillable nitrosamines.

Experimental

Apparatus and Reagents

(a) *Gas-liquid chromatograph*.—Varian Aerograph Model 1740-1 gas chromatograph equipped with two 9' × 1/8" od stainless steel columns packed with 15% Carbowax 20M-TPA on 60-80 Gas-Chrom P, conditioned overnight at 180°C, and installed for on-column injection. Detectors: standard flame ionization (FID) and an alkali flame ionization (AFID). Flow conditions: FID—He 47, H₂ 40, and air 400 ml/min. AFID—He 47, H₂ 63, and air 240 ml/min. Helium flow was monitored continuously with Matheson Model LF 100 mass flowmeter. (Mass flowmeter is direct reading instrument which requires no ambient temperature correction from 40 to 200°F and no gas pressure correction from 0.1 psia to 250 psia.) Flow diverters were installed in gas chromatograph to permit monitoring of hydrogen and air flows without disassembling detector. Hydrogen flow and, to a lesser extent, air flow were adjusted slightly from time to time in order to maintain desired detector sensitivity. Electrometer range used was 10⁻¹² amp/mv. Background current was not set at specific value but was kept in range of 32 × 10⁻¹² amp. Injector port and detector temperatures were 190 and 205°C, respectively. Column temperature was 115°C isothermal for routine analyses involving only DMNA.

(b) *N-Nitrosodimethylamine (DMNA) standard solution*.—Weigh 250 mg DMNA into 250 ml volumetric flask and dilute to mark with methylene chloride. Transfer 1 ml solution to 100 ml volumetric flask and dilute to mark with methylene chloride; dilute 12.5 ml of this solution to 100 ml in second volumetric flask to give final concentration of 1.25 µg/ml. Use 0.5 ml solution to fortify ham sample and for use as external standard.

(c) *2,3,5,6-Tetramethylpyrazine (TMP) internal standard solution*.—Weigh 94 mg TMP into 100 ml volumetric flask; dissolve in and dilute to mark with *n*-butanol. Transfer 1 ml of this solution to 10 ml volumetric flask and dilute to mark with *n*-butanol. Concentration of this solution is 0.094 µg/µl.

(d) *Solvents*.—Methylene chloride and methanol, distilled in glass; *n*-hexane, Mallinckrodt Nanograde.

(e) *Potassium hydroxide*.—Analyzed reagent grade.

(e) *Trifluoroacetic acid solution*.—0.2*N*. Dissolve 22.8 g trifluoroacetic acid in water and dilute to 1 L.

(f) *Sodium hydroxide solution*.—1.0*N*. Dilute 10 ml Baker standard 10*N* NaOH solution to 100 ml with water.

(g) *Sodium sulfate*.—Analyzed reagent grade, anhydrous, granular.

(h) *Glassware*.—(Numbers refer to Kontes Glass Company catalog unless otherwise indicated; equivalent glassware may be used.) (1) *Boiling flask*.—2 L single neck, 24/40 F , K-601000. (2) *Friedrich condenser*.—325 mm, 24/40 F , K-437000. (3) *Distilling head*.—24/40 F , K-517000. (4) *Distilling column*.—Plain 300 mm, 24/40 F , K-501000. (5) *Connecting adapter*.—24/40 F , K-167000. (6) *Liebig condenser*.—200 mm, 24/40 F , K-871000. (7) *Bent adapter*.—105°, 24/40 F , K-157000. (8) *Separatory funnel*.—250 ml with Teflon stopcock and stopper, K-636030. (9) *Büchner funnel*.—60 ml coarse porosity fritted glass disk, K-955000. (10) *Kuderna-Danish evaporative concentrator*.—240 ml, 24/40 F top, 19/22 F bottom, K-570001. (11) *Snyder column*.—3-Chamber, 150 mm, 24/40 F , K-503000, size 121. (12) *Micro Snyder column*.—1-Chamber, 19/22 F , K-569001. (13) *Concentrator tube*.—4 ml graduated, 19/22 F , K-570050, size 425. (14) *Pennyhead stopper*.—19/22 F , K-850500. (15) *Volumetric flasks*.—Low Actinic, 250 ml; 100 ml with F stoppers (Corning 55640). (16) *Erlenmeyer flask*.—Low Actinic, 1 L with F stopper (Corning 55020). (17) *Syringe*.—Glass, 10 μ l Hamilton 701-N.

(i) *Silicone oil baths*.—105 \pm 5°C and 50 \pm 2°C.

Sample Preparation

Digestion and distillation.—Digestion and distillation procedures were as described by Howard *et al.* (6). Four hundred g commercial ham was utilized for digestion and $\frac{1}{16}$ of the total digest (equivalent to 25 g ham) was distilled.

Extraction and cleanup.—Wash 50 ml distillate collected in 250 ml separatory funnel 2 min with 50 ml hexane by gently rocking solution to minimize emulsion formation. After layers separate, drain lower layer into 250 ml separatory funnel. Repeat hexane washing and separation. Add 0.2*N* trifluoroacetic acid (ca 6 ml) to aqueous layer to give pH 3. Extract 3 times with 50 ml methylene chloride, using a 3 min shake. To the combined methylene chloride extracts add 50 ml 1*N* NaOH. Shake 3 min and let separate. Filter methylene chloride layer through 35 g anhydrous sodium sulfate (prewet with methylene chloride) in 60 ml coarse fritted glass funnel into Kuderna-Danish evaporator with 4 ml concentrator

attached. Wash remaining aqueous alkaline layer $\frac{1}{2}$ min with 30 ml methylene chloride; then filter through sodium sulfate into Kuderna-Danish apparatus. Add 3 carborundum grains and insert a 3-section Snyder column (prewet with methylene chloride). Carefully concentrate sample to ca 3 ml by using 100–110°C silicone oil bath, immersing tube to ca 4 ml mark. Remove concentrator from oil bath; let cool to allow solvent held up in column to drain into tube. Attach micro Snyder column to concentrator tube. Concentrate solvent to 0.5 ml by partially immersing concentrator tube in 50°C water bath, occasionally lifting tube from bath to allow solvent in column and on walls to drain back into concentrator tube.

Gas Chromatographic Analysis

Four μ l internal standard TMP solution was added to 0.5 ml portions of methylene chloride solution of both standard DMNA and ham sample (10–25 ppb DMNA) and mixed thoroughly. An 8 μ l sample was injected into the gas chromatograph. The presence and quantity of DMNA in the ham sample were determined by comparing peaks with those of the standard. Both DMNA and TMP give symmetrical peaks. Peak heights were measured in standard and sample chromatograms from baseline drawn tangent to the bottom of the curves.

Results and Discussion

The DMNA concentration in cured ready-to-eat ham products obtained from local markets was determined by the procedure described in this paper. Of the 10 hams studied, 7 were different varieties of products from local and large domestic producers, including regular smoked hams as well as canned hams. Three (Dutch and Polish) imported hams were also analyzed; one of these had been treated at high cooking temperature. The chromatograms of all of the hams contained either no peaks or very small peaks with retention times in the general area of that of DMNA. The identity of the latter peaks could not be established, but should they be DMNA, the levels in the samples would be less than 1 ppb, which is below the limit of sensitivity of the method. Typical chromatograms of a commercially processed ham with and without fortification with DMNA and showing the internal standard peak are given in Fig. 1. The peak eluting at about 6.0 min in the unfortified sample illustrates the very small peaks in the area of DMNA discussed above.

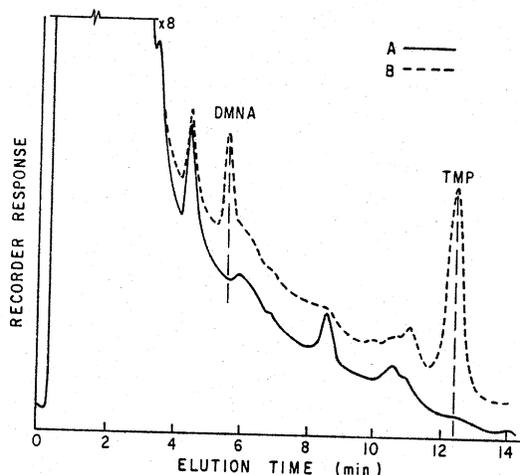


FIG. 1—Gas chromatogram of: A, commercially processed ham; B, commercially processed ham fortified with 25 ppb DMNA and TMP internal standard.

The background peaks present other than the DMNA and internal standard may be nitrogen-containing compounds of low basicity which are not removed in the acid treatment or large concentrations of compounds containing no nitrogen which overcome the selectivity of GLC detector response. The practical limiting concentration for the determination of DMNA by this procedure is 25 ppb. Satisfactory recoveries of DMNA added at this level ranged from an average of 73 to 100% as indicated in Table 1. The recovery values were corrected for apparent DMNA content, using an unfortified sample.

The simplicity and sensitivity of this method were made possible by the use of an alkali flame ionization detector. These detectors are known to be capable of extreme sensitivity but have erratic reproducibility characteristics and are very sensitive to operating conditions, the most critical of which is hydrogen flow (14).

It was found that during the course of the day the sensitivity of the detector declined approximately 20%. When the AFID was allowed to cool overnight, it regained a considerable amount of sensitivity. By manipulation of hydrogen and air flow rates most of the sensitivity which had been lost could be recovered.

Another problem with the use of the AFID is the assay solvent. Halogenated hydrocarbons are not usually recommended as solvents because of excessive detector recovery time (16). However,

Table 1. Recoveries of DMNA added to ham at a level of 25 ppb^a

Sample	Recovery, %	
	Range	Av.
Cooked and Smoked Ham ^b		
1	86-101	92
2	90-110	97
3	79-99	87
4	74-82	79
5	74-88	83
Canned Hams ^c		
6	90-98	94
7	84-89	86
8	76-91	85
9	67-80	73
10	98-103	100

^a Added to 3 samples of each ham.

^b Sample 4 was a ham cured in a special cure process and Sample 5 was a picnic ham.

^c Samples 7, 8, 9, and 10 were Dutch, Polish, nonperishable Dutch, and picnic hams, respectively.

methylene chloride was used in the assay in spite of this, since it facilitated sample cleanup (DMNA is readily soluble) and since its low boiling point simplified concentration of the solution.

To overcome the problems associated with the variability in sensitivity, an internal-external standard ratio procedure was utilized, permitting indirect comparison of the standard and ham samples. The same amount of TMP is used as internal standard in both the ham and standard solutions in order to normalize the DMNA peak height in the ham sample. This is done by multiplying the DMNA-to-TMP ratio in the standard by the TMP peak height in the ham sample. The actual peak height of DMNA is then divided by this value in order to obtain the per cent recovery. Standard and sample solutions are prepared in the same way. The detector was found to give a linear response over a range of 4-80 ng, which is within the limits of the assay.

2,3,5,6-Tetramethylpyrazine (TMP) was selected as an internal standard for this method because it is chemically nonreactive and soluble in methylene chloride, and gives a satisfactory elution time where there are no interfering peaks in the ham sample. More importantly, the TMP-to-DMNA detector response is constant with the variation in sensitivity of the detector. Experiments ensured that at the 25 ppb level of DMNA the TMP could be added reproducibly.

Studies are continuing to determine the applicability of this procedure to the quantitative determination of DMNA in other meat products.

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Mention of commercial names does not imply endorsement by the U.S. Department of Agriculture.