Use of Vacuum Distillation and Gas Chromatography–Mass Spectrometry for Determination of Low Levels of Volatile Nitrosamines in Meat Products

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A method is described for the identification and determination of volatile nitrosamines in meat products based on vacuum distillation linked with gas chromatography–mass spectrometry. Lower limits of detection for the technique range from 25 ppb for *N*-nitrosodimethylamine (DMN) to 65 ppb for *N*-nitrosodipentylamine. The technique has been used to screen seven types of meat products for the presence of volatile nitrosamines, and all results were negative. The paper also discusses techniques by which certain individual nitrosamines, including DMN, can be detected at levels of <10 ppb.

Since Magee and Barnes (1956, 1967) published their work on the carcinogenic properties of nitrosamines, numerous authors claim to have identified nitrosamines in a wide range of foodstuffs. These included smoked fish (Ender and Ceh, 1968), raw and cured meats (Mohler and Mayrohfer, 1968), flour (Kroller, 1967), milk (Hedler and Marquardt, 1968), and spirits (McGlashan *et al.*, 1968). In particular, efforts have been made to find them in cured meats where, theoretically, naturally occurring secondary amines might react with nitrite to form nitrosamines.

A wide range of analytical techniques was used (Fishbein and Falk, 1969) including nitrite release (Daiber and Preussmann, 1964), polarography (Heath and Jarvis, 1955; Lydersen and Nagy, 1967), tlc (Preussmann *et al.*, 1964) and glc (Serfontein and Hurter, 1966). Many of these methods are open to criticism and several recent studies (Heyns and Koch, 1970; Foreman *et al.*, 1970; Howard *et al.*, 1970; Henriksen, 1970) indicate that many, if not all, of these methods may lack the necessary specificity and/or sensitivity to give reliable results. It follows that if the methods themselves are suspect, the results thus obtained are also suspect. These doubts and criticisms are disturbing, in view of the significance which has been placed on the presence of small amounts of nitrosamines in foodstuffs (Druckrey *et al.*, 1963).

Early in our studies we examined samples of luncheon meat for their volatile nitrosamine content, using a steam distillation cleanup by ion-exchange chromatography and determination by gas chromatography with flame ionization detection. The type of chromatogram obtained is shown in Figure 1. From retention times we could presume the presence of significant amounts of several nitrosamines, but when this extract was examined by gas chromatography–mass spectrometry (gc–ms), we found that the peaks which might be ascribed to nitrosamines on the basis of retention times alone were, in fact, due to a range of compounds such as terpineol, benzaldehyde, and nonanol, and that no nitrogen-containing compounds were present.

To evaluate the many claims for nitrosamines in foodstuffs, we developed a determinative technique using gc–ms and applied it to the determination of volatile nitrosamines in meat products.

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EXPERIMENTAL

IMPORTANT SAFETY NOTE: Nitrosamines are highly carcinogenic compounds and all experimental work should be done in a well ventilated area. Safety gloves should be worn whenever nitrosamines are being handled.

Apparatus. A Pye 104 gas chromatograph was used, fitted with a 5.5-m × 4-mm i.d. glass column packed with 10% Carbowax 20M on acid washed, silanized Celite (100–120 mesh) and temperature programmed from 100° to 200° C at a rate of 2° C per min. Helium, at a flow rate of 40 ml per min, was used as the carrier gas in the gc–ms determinations. In all other gas chromatography work, nitrogen was used. Injections were made directly on to the column.

The column effluent was split approximately 10:1, the larger amount passing to an A.E.I. MS 902 mass spectrometer by means of a fritted glass separator (Watson and Biemann, 1965), and remainder to a flame ionization detector.

Fragment ions of composition NO^+ were monitored by using the peak matching facilities of the mass spectrometer, the mass scale being calibrated by reference to the background O_2^+ ion. The NO^+ ion current was recorded with a Brüel and Kjaer Level Recorder (Type 2305).

Compounds studied were the *N*-nitroso derivatives of dimethylamine (DMN), *N*-methylethylamine (MEN), diethylamine (DEN), *N*-methylisopropylamine (Mi–PN), *N*-methylisobutylamine (Mi–BN), *N*-methylbutylamine (Mn–BN), diisopropylamine (Di–PN), dipropylamine (Dn–PN), *N*-ethylbutylamine (En–BN), diisobutylamine (Di–BN), dibutylamine (Dn–BN), *N*-methyloctylamine (MON), diisopentylamine (Di–Pent N), dipentylamine (Dn–Pent N), pyrrolidine (NNPyrr), and piperidine (NNPip).

Analytical Procedure. Weigh 250 g of mixed sample into the container of a macerator, add 50 g of sodium chloride, 10 g of potassium carbonate, and 250 ml of water, and macerate the mixture for 10 min. Transfer the resultant suspension to a 2-l. round-bottomed flask, washing out the macerator with a minimum of water. Immerse the flask in a water bath at <10° C and connect it as part of a vacuum still, passing ice water through the condenser and cooling the 500-ml receiver in an ice bath. Apply the maximum vacuum of a rotary oil pump and heat the water bath to 65° C, ensuring that the whole surface of the distillation flask is kept at this temperature. Maintain these conditions until no more distillate is produced (3–5 hr). Release the vacuum and wash the condenser and adaptors with water, adding the washings to the distillate. Note the volume of distillate (≮250 ml). Extract the distillate and washings with two 250-ml portions

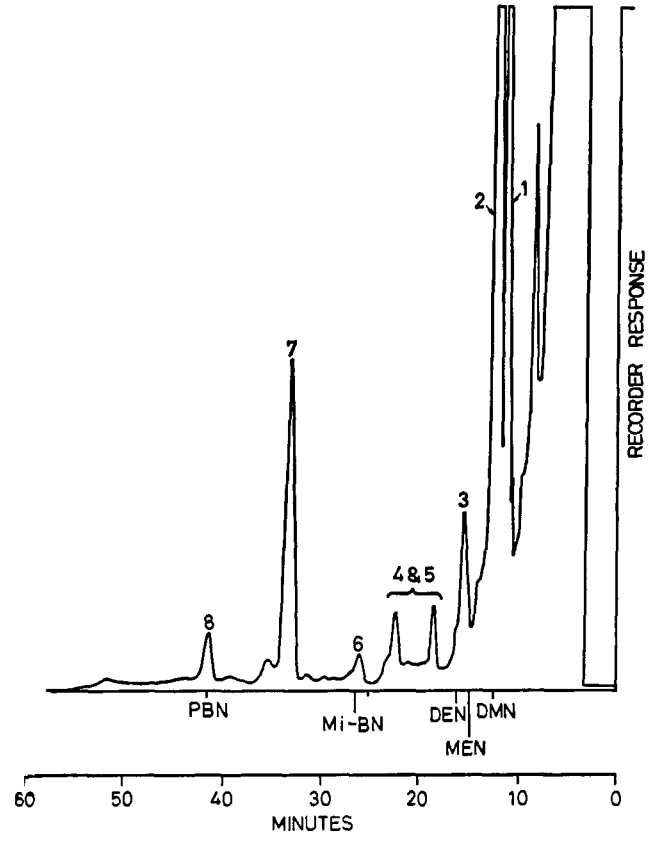

Figure 1. Gas chromatography of pork luncheon meat extract prepared by steam distillation and ion exchange

Instrument: Pye 104 F.I.D. Column: 5 ft 10% Carbowax 20M on acid washed HMDS treated Celite. Temperature: Isothermal at 170° C. Carrier gas: Nitrogen. Inlet pressure: 28 psi. Flow rate: Approximately 36 ml/min. Peak 1, acetoin; 2, hydroxy acetone; 3, nonanol; 4 and 5, terpene $C_{10}H_{18}O$; 6, benzaldehyde; 7, sesquiterpene and furfuryl alcohol; 8, α-terpineol. The corresponding retention times of nitrosamines are indicated

of dichloromethane, running the dichloromethane extracts into a Kuderna–Danish evaporator.

Immerse the lower part of the evaporator in a water bath held at 50° C, and evaporate the dichloromethane, making sure that the level of solution in the evaporator is always above the water level in the bath.

When the volume of the extract is approximately 5 ml, remove the bulb from the collection tube, fit a Quickfit cone to the tube, and continue evaporating until the volume is reduced to 1 ml. Add 0.4 ml of redistilled hexane and continue the concentration until the volume is 0.1 ml.

Examine 10 μl of this concentrated extract by high-resolution gc–ms with the mass spectrometer set to detect the NO^+ ion mass (29.99799) at a resolution of 15,000.

Note those peaks in the chromatogram which give a positive NO^+ response. If a positive result is obtained, inject an additional 10-μl sample and record the low resolution mass spectrum of the corresponding peaks in the conventional way.

High-resolution mass spectrometry monitoring for NO^+ ions will detect a minimum of 0.1–0.2 μg of individual nitrosamines. An identifiable low-resolution mass spectrum can be obtained from 0.05 μg of a single pure nitrosamine injected on the column, but in the presence of other species distilled from meats by the above technique, a minimum of 0.5 μg is required to give a sufficiently intense spectrum for positive identification. For a 10-μl injection this corresponds to a level of 20 ppb of individual nitrosamines in the original sample, assuming 100% recovery through the distillation and extraction procedures.

But tests made on the nitrosamines listed above using the procedures resulted in recoveries which ranged from 92% for DEN and MEN to 31% for Dn-Pent N (Table I). Therefore the 20 ppb detection limit must be increased to 25 ppb for DEN and MEN and 65 ppb for Dn–Pent N.

Table I. The Percentage Recovery of Nitrosamines at the 10-μg Level from an Aqueous Salt Solution and from a 250-g Sample of Ham Using Steam Distillation and Vacuum Degassing Without Cleanup

	From aqueous salt soln		From 250-g ham	
Nitrosamine	Steam distillation	Vacuum degassing	Steam distillation	Vacuum degassing
DMN	86	88	*a*	*a*
MEN	92	82	46	92
DEN	93	87	88	93
Mi-PN	93			87
Mi-BN	97			*a*
Mn-BN	93	89	80	85
Di-PN	96	89	67	89
Dn-PN	96		64	86
En-BN	91	90	70	86
Di-BN	88		53	78
Dn-BN	91	84	44	
MON	80	54	22	33
Di-Pent N	98			47
Dn-Pent N	46	57	31	31
NNPyrr	21	79	41	44
NNPip	76	90	33	73

[a] Interference from naturally occurring materials. All results represent the mean value for at least ten determinations.

Table II. Percentage Recoveries of 10 μg Nitrosamine through Various Stages of the Proposed Method

			% Recovery	
Nitrosamine	Carbon no.	Ion exchange	To 1.0 ml in dichloromethane	To 0.1 ml in hexane
DMN	2	94	93	60
MEN	3	97	89	89
DEN	4	94	97	81
Mi-PN	4	89	101	
Mi-BN	5	92	105	
Mn-BN	5	91	95	91
Di-PN	6	95	90	85
Dn-PN	6	84	96	84
En-BN	6	84	91	91
Di-BN	8	79	97	87
Dn-BN	8	72	95	86
MON	9	25	98	98
Di-Pent N	10	63	110	
Dn-Pent N	10	63	110	
Dn-Pent N	10	20	102	102
NNPyrr	5	68	98	92
NNPip	6	91	90	82

All values represent the mean value of at least six determinations.

RESULTS AND DISCUSSION

Development of the Method. CLEANUP OF DISTILLATES. The three main column cleanup techniques which are quoted in the literature for nitrosamine extracts are based on alumina (Sen *et al.*, 1970), ion exchange (Sen *et al.*, 1969) or acid–Celite (Howard *et al.*, 1970). Alumina has only been used as an extra cleanup step after ion exchange and the sulfuric acid–Celite technique proposed by Howard is only suitable for DMN and MEN.

We tried a mixed Amberlite IR 120/IRA 407 column as a cleanup step. As the recoveries (Table II) of 10-μg quantities

of individual nitrosamines show, appreciable losses occur for dialkyl nitrosamines with a carbon number greater than 8 as well as for NNPyrr. Since our aim was to develop a screening method for as many volatile nitrosamines as possible, we decided to develop a technique which did not include a cleanup stage.

THE USE OF STEAM DISTILLATION. Steam distillation can be used in place of vacuum distillation and is quicker, but the recoveries of added nitrosamines are generally much lower, as shown in Table I, than those from vacuum distillation. These lower recoveries mean lower sensitivity.

RECOVERIES OF NITROSAMINES THROUGH DISTILLATION AND PARTITION CONCENTRATION STEPS. When aqueous solutions of nitrosamines are extracted once with an equal volume of dichloromethane, more than 95% is found in the organic phase except for DMN, where only 77% is transferred to the organic phase. However, by partitioning with two portions of dichloromethane, less than 5% of the DMN is left in the aqueous phase.

Losses of nitrosamines during concentration from 500 ml of dichloromethane solution to 1 ml, transfer to hexane, and concentration to 0.1 ml are given in Table II. The only significant loss is that for DMN in the final concentration step.

Recoveries through the whole process as far as concentration to 1 ml are given in Table I. For recoveries from meat we used samples of cooked ham as our base material. We found that the background due to this product on the chromatographic scans was sufficiently low for an accurate assessment of recoveries to be made for all the nitrosamines except DMN and Mi-BN.

When recoveries of the higher nitrosamines from ham slurries and from an aqueous solution of NaCl and K_2CO_3 are compared, it can be seen that recoveries are much lower from the former than from the latter. It is not known whether these higher nitrosamines are decomposed or whether they are merely not extractable using the techniques employed.

THE USE OF GC–MS AS THE DETECTION STAGE. If each member of a class of compounds gives rise to an ion in its mass spectrum which is unique to that class, then by setting the mass spectrometer to monitor for the unique ion only, the mass spectrometer becomes a specific detector for that class of compound. Previous uses of the fixed mass technique in combination with gc–ms have been mainly confined to low-resolution mass spectrometry (Henneberg and Schomburg, 1971).

Each of the standard nitrosamines available for examination contained an ion of *m/e* 30 in its mass spectrum and high-resolution mass spectrometry established that this ion was composed of the species NO^+ and CH_4N^+. We decided to use the NO^+ ion as the fixed mass in preference to the CH_4N^+ ion, as the latter is a prominent ion in the spectra of many amino compounds (Budzikiewicz *et al.*, 1967), whereas NO^+ ions arise only from compounds containing NO or NO_2 groups.

The more probable combinations of C, H, O, and N of mass 30 and the resolution required to separate each combination from NO are given in Table III. A resolution of 15,000 is sufficient to separate the species NO^+ from all others, with the exception of $C^{18}O^+$. As we were aiming to identify nitrosamines at the lowest possible level, it was decided that, although a resolution of 25,000 is attainable with the MS 902 mass spectrometer, the resultant loss of sensitivity is too great for this particular application. Therefore the extracts prepared by the procedures already described were examined for the presence of NO^+ ions at a resolution of 15,000.

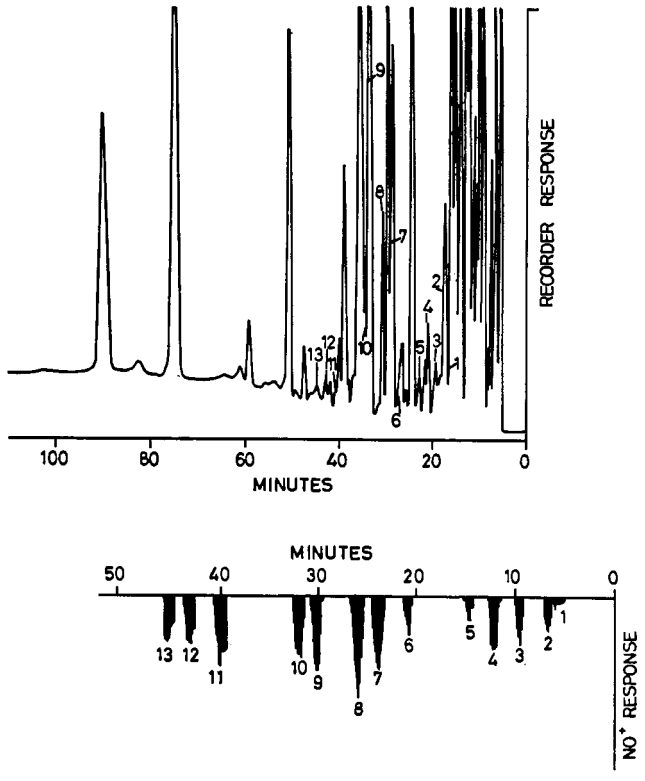

Figure 2. Chromatogram and corresponding NO^+ detector trace from pork luncheon meat spiked with nitrosamine. Peak numbers as in Table IV

Table III. Possible Elemental Compositions of Mass 30 and Resolution Required to Separate from NO^+

Composition	Exact mass	Approximate resolution to separate NO^+
NO	29.9980	
$C^{18}O$	29.9992	25,000
^{15}N	30.0002	13,600
^{13}CHO	30.0061	3,700
CH_2O	30.0106	2,400
H_2N_2	30.0208	1,300
$CH_3{}^{15}N$	30.0236	1,200
$^{13}CH_3N$	30.0309	900
CH_4N	30.0344	800
$^{13}CCH_5$	30.0425	700
C_2H_6	30.0469	600

The sensitivity of the gc–ms combination was determined in the following way. Standard nitrosamines were added at the 5-μg level to the dichloromethane extract prepared from a sample of pork luncheon meat after reduction to 1-ml volume. The mixture was then concentrated to a volume of 0.1 ml in hexane. Figure 2 shows the chromatogram and corresponding NO^+ ion record for this sample. Low-resolution gc–ms of the numbered peaks resulted in the identification of all the added nitrosamines as shown in Table IV. The success of the method is demonstrated by the location and identification of the nitrosamines which were not resolved from major components of the mixture. Although Mn-BN and Dn-PN were not resolved under the gas chromatographic conditions used, both were identified as components of the mixture. Similarly, Di-BN and En-BN were not resolved but were successfully identified. Peaks 1, 5, 6, and 9, which also gave a positive response, were found

Table IV. Compounds Identified in Spiked Pork Luncheon Meat Extract

Peak	Compound
1	Monoterpene hydrocarbon, hydroxyacetone
2	DMN
3	MEN, monoterpene hydrocarbon
4	DEN
5	Silicone, two oxygenated compounds
6	Oxygenated monoterpene
7	Dn-PN, Mn-BN, sesquiterpene hydrocarbon
8	Di-BN, En-BN, oxygenated monoterpene
9	Oxygenated monoterpene
10	Pn-BN, sesquiterpene hydrocarbon
11	Dn-BN
12	NNPip
13	NNPyrr

not to contain nitrosamines. As the compounds responsible for these peaks are oxygenated and do not contain nitrogen, their response is attributed to $C^{18}O^+$ and not to NO^+ ions. The peak due to hydroxyacetone is particularly interesting. In the presence of an excess of this compound it is very difficult to identify trace amounts of DMN by low-resolution mass spectrometry alone since their retention times are almost the same, their molecular weights are the same, and their fragmentation patterns are similar.

Certain specific nitrosamines can be detected at a sensitivity of 10 ppb or better by monitoring for ions other than NO^+. If it is suspected that, *e.g.*, DMN is present in the extract, the mass spectrometer under high-resolution conditions is set to record only the mass corresponding to the molecular ion of DMN. As this is approximately five times more abundant than NO^+ ion, a fivefold gain in sensitivity is achieved. Similarly, a gain in sensitivity will be realized for any nitrosamine which has a more abundant molecular ion than NO^+ ion. The greatest gains are given by DMN, DEN, MEN, Mi-PN, NNPyrr, and NNPip.

Monitoring the molecular ion has the additional advantage that if a positive result is found it need not be confirmed by recording a low-resolution mass spectrum, as it is exceedingly unlikely that any other compound of the same retention time as the nitrosamine will contain a fragment ion in its mass spectrum which corresponds to the chemical composition of the nitrosamine molecular ion.

It is not necessary to perform a separate run for every nitrosamine suspected to be present, provided that there is sufficient time between the elution of the nitrosamines from the gas chromatograph to allow the mass spectrometer to be readjusted to detect the next molecular ion.

All low-resolution mass spectral data obtained during this study have been submitted to the Mass Spectrometry Data Centre, A.W.R.E., Aldermaston, Berks, U.K.

Application to Meat Samples. The NO^+ ion monitoring technique described above has been used to examine a number of commercial meat products for the presence of volatile nitrosamines at the levels described in the method.

Products examined were canned pork luncheon meat, Danish back bacon, and injection-cured and slice-cured English bacon, all of which were cured with nitrate and nitrite. Other products examined were nitrite-cured cooked ham, fresh pork, and fresh beef.

In all cases we failed to detect the presence of any nitrosamine at the levels claimed for this method (*i.e.*, 25–65 ppb) depending on the nitrosamine.

These results are clearly at variance with a number of published claims to have identified nitrosamines in meat products such as raw beef (0.25 ppm of DMN) (Mohler and Mayrohfer, 1968).

Although this technique will not allow the analyst to prove the complete absence of nitrosamines in meat products, it does give a means of proving or disproving claims of the presence of volatile nitrosamines in the ppm rather than ppb range.

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