Mass Spectrometric Quantification of Traces of Volatile *N*-Nitrosamines in Meat Products

R. W. Stephany,* J. Freudenthal, E. Egmond, L. G. Gramberg, and P. L. Schuller

A combined gas chromatographic–mass spectrometric method for the detection and quantification of five volatile *N*-nitrosamines in meat products is described. The *N*-nitrosamines are isolated from spiked samples, purified, and concentrated by steam distillation, dichloromethane extraction, and evaporation. The components of the resulting extract are gas chromatographically separated under isothermal conditions on both an apolar and a polar glass capillary column and detected by mass fragmentography at a resolution of approximately 4000. Recoveries from various meat products are determined for 10 and 50 ppb (micrograms per kilogram) of *N*-nitrosamines: *N*-nitrosodimethylamine (70–101%), *N*-nitrosodiethylamine (78–99%), *N*-nitrosodi-*n*-butylamine (68–100%), *N*-nitrosopyrrolidine (40–62%), and *N*-nitrosopiperidine (78–103%). The lower detection and quantification limit of the method described appeared to be 0.1–0.2 ppb depending on the *N*-nitrosamine studied. None of the *N*-nitrosamines mentioned above could be detected in the unfortified samples (n = 9).

Recent work from various laboratories has established that trace amounts of carcinogenic *N*-nitrosamines are present in certain types of fish and meat products used for human consumption (Sen, 1972; Crosby et al., 1972; Fazio et al., 1973; Panalaks et al., 1973; Fong and Chan, 1973). Among the numerous analytical techniques used for the identification and quantification of *N*-nitroso compounds (Schuller, 1972; Du Plessis and Nunn, 1973) gas–liquid chromatography (GLC) and mass spectrometry (MS) provide the best methods for the characterization of *N*-nitrosamines in food products. However, even these sophisticated methods have their serious drawbacks. With conventional gas chromatography using a flame ionization detector the *N*-nitrosamine containing extract has to be free from extraneous matter of similar retention time. In practice this is very difficult to achieve and the use of nitrogen-selective detectors, such as for instance the thermionic detector (Palframan et al., 1973; Riedmann, 1974) and the Coulson conductometric detector (Palframan et al., 1973), is recommended. Fragment ions corresponding to the trimethylsilyl group were shown to be responsible for false positive results during the analysis of food extracts with exclusive mass spectrometric methods (Dooley et al., 1973).

Although it is accepted that to date the most reliable procedure for the unambiguous detection of traces of *N*-nitrosamines is a combination of gas chromatography and high-resolution mass spectrometry (GC–MS), still there are various other possibilities for performing such an analysis. For instance, Telling et al. (1971, 1973) and Bryce and Telling (1972) described a single packed column, temperature programmed, NO^+ ion as well as a specific ion monitoring technique, whereas Gough and Webb (1972) and Crosby et al. (1972) described a single packed column, isothermal, parent ion monitoring technique. On working with standard solutions exclusively, Heyns and Röper (1971) tested a dual capillary column, temperature programmed, total spectrum monitoring technique. Gough and Webb (1973) developed, on working with standard solutions, a dual packed column, pressure-programmed, peak cutting, parent ion monitoring technique. Recently, Heyns et al. (1974) reported a single capillary column, temperature programmed, total spectrum monitoring technique for the analysis of the reaction products of the Maillard reaction.

In this study a dual capillary column, isothermal, single ion monitoring high-resolution technique will be described applicable to the analysis of meat products.

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EXPERIMENTAL SECTION

Samples. Most of the samples were purchased from local stores. The minced meat dough (45% pork, 45% beef, and 4% water) was a sterilized intermediate in the production of canned complete meals. Luncheon meat I (52% pork, 28% beef, and 12% water) was a specially processed pasteurized control sample used in toxicologic studies at our Institute (Van Logten et al., 1972). Frying fat was the remaining part of a vegetable fat used to fry approximately 250 meatballs ("Dutch frikadel" type) per kg of fat.

Apparatus and reagents used included: SVL steam distillation equipment, 2000 ml (Sovirel); Kuderna-Danish evaporative concentrator K 570000, 250 ml, with calibrated lower tube, 10 ml (Kontes Glass Co.); Reacti-vial, 1 ml (Pierce Chemical Co.); Varian 1740 gas chromatograph equipped with either an apolar or a polar all-glass capillary column: OV-101, i.d. 0.45 mm, length 24 m, and Ucon 50 HB-5100, i.d. 0.45 mm, length 30 m, respectively; and a Varian Mat CH 5 single focusing mass spectrometer. *N*-Nitrosamines were obtained from the following sources and were used without further purification: *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine, K&K Laboratories, Inc.; *N*-nitrosodi-*n*-butylamine and *N*-nitrosopiperidine, Schuchardt, Munchen; and *N*-nitrosodiethylamine, Eastman. Antifoam "S" tablets were purchased from Thompson and Capper Ltd., Liverpool. Dichloromethane (Baker) was freshly distilled before use. All other reagents were of analytical grade quality and were used without further purification.

Isolation and Concentration Procedure. The method used to isolate and concentrate the volatile *N*-nitrosamines is outlined in Scheme I. This procedure is practically the same as that described by Cox (1973). If immediate subsequent GC–MS analysis of the extract was not possible, the reacti-vial with extract was stored at −70 °C until further use.

The recoveries of the different *N*-nitrosamines in the extraction procedure were obtained from three extracts (see Scheme II): (1) the extract from a meat sample spiked *before* the extraction procedure with a known amount of

Table I. Mass Spectrometric and Gas Chromatographic Data Used for the Quantification of Various *N*-Nitrosamines

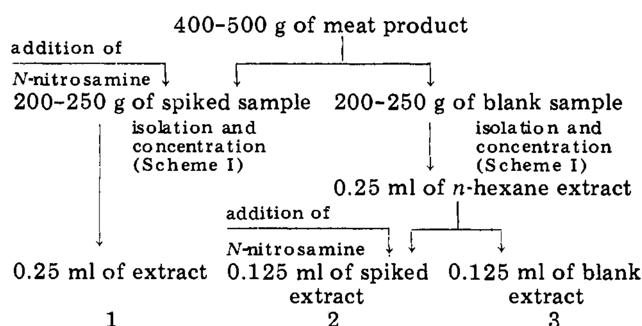
Compound	Bruto formula	*m/e*[a]	Ucon 50 HB-5100 column: Retention time[b]	Ucon 50 HB-5100 column: *T*, °C	OV-101 column: Retention time[b]	OV-101 column: *T*, °C
N-Dimethylnitrosamine	$C_2H_6N_2O$	74	2 min 47 s	70	1 min 42 s	100
N-Diethylnitrosamine	$C_4H_{10}N_2O$	102	4 min 48 s	70	2 min 20 s	100
N-Di-*n*-butylnitrosamine	$C_8H_{18}N_2O$	158	6 min 20 s	110	5 min 49 s	125
N-Nitrosopyrrolidine	$C_4H_8N_2O$	100	5 min 04 s	70	2 min 40 s	125
N-Nitrosopiperidine	$C_5H_{10}N_2O$	114	4 min 40 s	110	2 min 26 s	140

[a] The *m/e* values are the molecular peak values. [b] The retention times proved to be reproducible within a few thousand provided the column temperatures are kept carefully constant.

Scheme I. Isolation and Concentration Procedure for the Analysis of *N*-Nitrosamines in Meat Products

200–250 g of minced sample —(steam distillation)→ 400 ml of neutral distillate —(acidifying, dichloromethane extraction)→ 160 ml of dichloromethane extract —(alkaline washing, evaporative concentration)→ 2 ml of dichloromethane extract —(*n*-hexane addition, evaporative concentration)→ 0.25 ml of *n*-hexane extract

Scheme II. Experimental Arrangement for the Determination of Recovery Rates of *N*-Nitrosamines from Meat Products



N-nitrosamine (extract 1); (2) the extract of a blank meat sample spiked *after* the extraction procedure with the same amount of the same *N*-nitrosamine (extract 2); (3) the extract of a blank meat sample (extract 3). Quantitative determinations done in these three extracts will give recoveries for the extraction procedure as well as the amount in the blank sample.

GC–MS Procedure. For the quantitative determinations the mass fragmentographic method is used (Hammar and Hessling, 1971; Freudenthal and Greve, 1973). This means that the mass spectrometer serves as a selective and sensitive detector by tuning it to a characteristic mass of the substance under consideration. In this method the substance is characterized by one or more masses and its gas chromatographic retention time. Polar and apolar capillary columns were chosen for these measurements because of their high resolution and selectivity. The separator used for the coupling of the gas chromatograph and mass spectrometer was an all-glass jet separator suitable for the capillary columns.

In mass fragmentography several general methods can be used and were tried by us.

(1) **The Single Peak Method at Low Resolution.** In this method the substance is characterized by one peak and the retention time. It is applicable in many instances for substances with a high molecular weight. For the lightweight and volatile *N*-nitrosamines described here, this method is not specific enough. Too many fragment or molecular ions are present in the low mass range region and too high a resolution has to be used at the cost of the sensitivity (compare 4c) to avoid false positive results.

(2) **The Multiple Peak Method at Low Resolution.** The selectivity has increased in this method compared to that mentioned under (1) by using more characteristic peaks. Unfortunately, the intensity of other peaks in, for example, the spectrum of *N*-dimethylnitrosamine is too low to bring us in an interesting sensitivity range.

(3) **The Single Peak Method at Low Resolution with More than One Column.** Compared to method (1) the selectivity is increased by using more than one capillary column. An apolar as well as a polar column were used, such that the characteristics of the columns were quite far apart. This method was a promising one, but the ratio between the signal and the background was not acceptable in several cases.

(4) **The Single Peak Method at Medium to High Resolution with More than One Capillary Column.** In the foregoing methods either the signal to background ratio was insufficient or false positives were observed. For this reason a mass fragmentographic method was developed with a sufficient high selectivity. This selectivity is mainly determined by the following factors.

(a) *The Resolution of the GLC Column.* Capillary columns with a high resolution were used to separate peaks situated closely together. With increasing resolution of the various columns used, more peaks in the gas chromatogram were obtained with the same extract. However, one is interested only in a very small retention time range and the numerous peaks eluted outside this range can be ignored and do not interfere. Polar and apolar columns were used; none of them were silanized. Interfering silyl ions were not observed, even with the use of silicone-containing antifoam tablets during the isolation and concentration procedure. Examples of fragmentograms obtained under the conditions listed in Table I of 10 ppb of *N*-nitrosopiperidine in frying fat extracts chromatographed on the OV-101 column are given in Figures 1–4. These figures are for illustration purposes only; retention times were not determined from the GLC graph but with a stopwatch.

(b) *The Stability of the Retention Time of the Gas Chromatographic System.* Columns were used with a high stability in retention times and generally the overall stability of the whole GLC system was shown to be 3 per 10^3 or better. Such a stability can only be obtained under isothermal GLC conditions. As already mentioned before, the resulting reproducible retention times were determined with a stopwatch.

(c) *The Resolution of the Mass Spectrometer.* If the resolution of the mass spectrometer increases, the selectivity will increase too, but the sensitivity will decrease. For the reported cleanup procedure it was found that a resolution of about 4000 (10% valley) was a fair compromise between selectivity and sensitivity. Above this resolution the selectivity did not improve while the sensitivity decreased. It has to be mentioned that for other extraction procedures not reported in this paper other

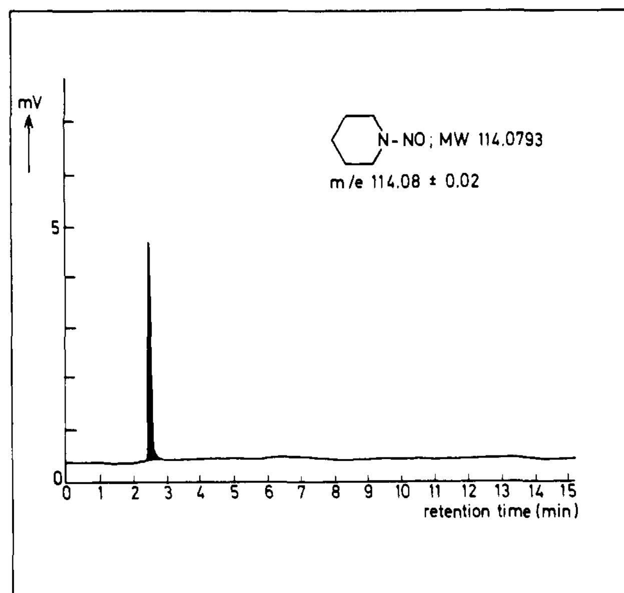


Figure 1. Chromatogram of 6 ng of *N*-nitrosopiperidine (*n*-hexane standard solution); detection, *m/e* 114.08 ± 0.02.

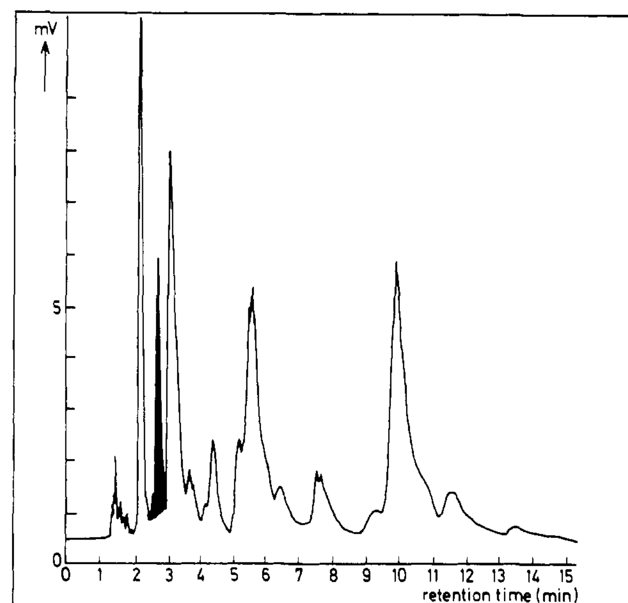


Figure 2. Chromatogram of extract 1 of 500 mg of spiked frying fat; detection, *m/e* 114.08 ± 0.02.

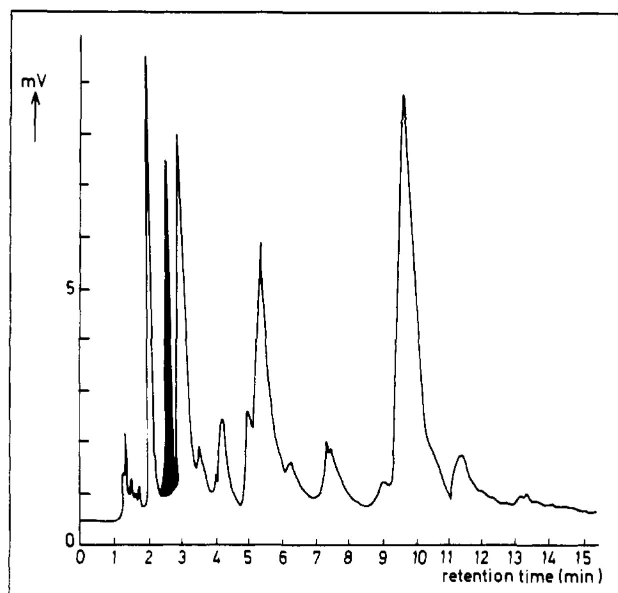


Figure 3. Chromatogram of spiked extract 2 of 700 mg of frying fat; detection, *m/e* 114.08 ± 0.02.

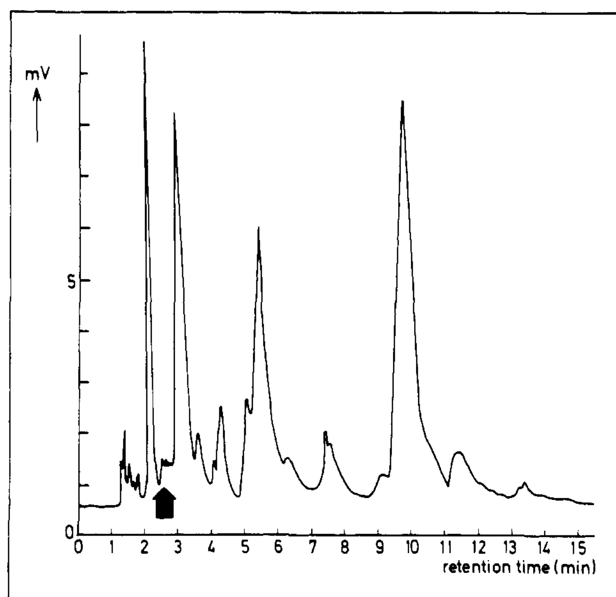


Figure 4. Chromatogram of blank extract 3 of 700 mg of frying fat; detection, *m/e* 114.08 ± 0.02.

optimum resolution settings for the mass spectrometer—sometimes in the range of 10000—were found. The sensitivity with a resolution of 4000 was suitable to quantitate 0.1–0.2 μg/kg of the various nitrosamines with an accuracy of about 30%.

Method 4 was chosen for the determination of the recoveries in the extracts of meat products. The amounts of nitrosamines found on the two different columns should agree of course. This agreement can be seen as an internal and additional check of the method. Quantitative disagreement between the results obtained with both columns indicates the presence of interfering substances. In this case for unequivocal confirmation positive results should be confirmed with high-resolution mass spectrometry (resolution 10000–12000), provided enough sample is available. However, with the GLC–MS system used by us now for 2 years false positive results were never found.

An apparent disadvantage of our method seems to be that one mass only can be monitored per GLC run.

Table II. Total Recovery of 50 ppb of *N*-Nitrosodimethylamine (DMN) from Various Samples of Meat Products

Sample	Recovery, %
Luncheon meat I	85
Luncheon meat II	70
Smoked Guelder sausage I	99

Nevertheless this was preferred because GLC conditions could be rigorously optimized and relatively short retention times could be maintained. Attempts to achieve a further shortening of the total time of analysis by application of multiple ion detection during one GLC run have not been made by us. Peak jumping at high resolution can very easily lead to instrumental errors.

RESULTS

The results of the recovery measurements for the different *N*-nitrosamines in various types of meat extracts

Table III. Total Recovery of 10 ppb of *N*-Nitrosodimethylamine (DMN), *N*-Nitrosodiethylamine (DEN), *N*-Nitrosodi-*n*-butylamine (DBN), *N*-Nitrosopyrrolidine (PYR), and *N*-Nitrosopiperidine (PIP) from Various Samples of Meat Products

Sample	Recovery, %				
	DMN	DEN	DBN	PYR	PIP
Minced meat dough I	92	97	82	48	103
Minced meat dough II	91	78	86	45	84
Fried minced meat	95	88	88	53	89
Smoked Guelder sausage I	70				
Smoked Guelder sausage II	98	83	68	40	78
Luncheon meat II	83				
Fried meat balls, "Dutch frikadel" type	96	89	98	49	87
Frying fat	101	99	100	62	99

are given in Tables II and III.

DISCUSSION

The recovery of *N*-dimethylnitrosamine was good within the range studied (10–50 ppb). At the level of 10 ppb the recovery for *N*-diethylnitrosamine, *N*-di-*n*-butylnitrosamine, and *N*-nitrosopiperidine was also determined and found to be equally as good as the recovery of *N*-dimethylnitrosamine. At the level of 10 ppb the recovery of *N*-nitrosopyrrolidine was in the range of 40–62%. A similar relatively lower recovery for *N*-nitrosopyrrolidine has been observed by others (Crosby et al., 1972; Bryce and Telling, 1972; Telling et al., 1975). In the blank samples none of the *N*-nitrosamines was detected indicating either their absence in the samples or that their concentration was lower than 0.2–0.1 ppb depending on the type of *N*-nitrosamine searched for.

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Quantitative Determination of Zinc, Iron, Calcium, and Phosphorus in the Total Diet Market Basket by Atomic Absorption and Colorimetric Spectrophotometry

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A simple method has been developed to determine zinc, iron, calcium, and phosphorus using the dry ash procedure of the AOAC (Association of Official Analytical Chemists, "Official Methods of Analysis", 12 ed, Washington, D.C., 1975) with a few minor modifications for 12 total diet market basket composites. The ash is diluted with 0.1 N HCl, made to a known volume, and analyzed for zinc and iron by atomic absorption spectrophotometry (AAS). A second series of dilutions is made from the original dilutions; then lanthanum is added so that each composite contains 1% lanthanum in 0.1 N HCl. These dilutions are then analyzed for calcium by AAS. A third series of dilutions is made from the initial dilutions and phosphorus is then determined by the molybdophosphoric acid colorimetric method (Halmann, M., *Anal. Chem. Phosphorus Compd.* **37** (1972)). The proposed method was applied in part to 45 total diet market baskets. Recoveries averaged between 94 and 99% for the four metals analyzed. Methods for the analysis of zinc, iron, calcium, and phosphorus appear in various scientific literature but no provisions are made for the analysis of these metals in combination in the total diet of man.

This paper describes a procedure for the analysis of zinc, iron, calcium, and phosphorus and may be applied to other metals as well, if the vapor pressure of the metal is not appreciable at the temperature given in this method for the dry ashing step. The total diet market basket composition is given in Table I. A total diet market basket represents the recommended 2-week diet of a 15–20 year old male or female for the region of the country in which it is collected.

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EXPERIMENTAL SECTION

Reagents and apparatus used included the following.