Rapid Solid-Phase Extraction Method for the Detection of Volatile Nitrosamines in Food

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A rapid solid phase extraction (SPE) method was developed to analyze volatile *N*-nitrosamines (NAm) in foods. The currently used vacuum distillation technique is time and solvent consuming and is cumbersome for multiple analyses. Our objective was to develop a robust analytical method to measure the eight most common volatile NAm found in foods. The extraction procedure was based on two consecutive extraction/concentration steps using Extrelut and Florisil SPE. The amounts of food sample and solvent required were reduced compared to the conventional vacuum distillation method without affecting the sensitivity which was at a 0.3 ppb detection limit. A greater confidence in the analyte detection was obtained by the use of a capillary GC column, instead of the conventionally used open packed column, prior to thermal energy analyzer (TEA) detection. Another advantage of our method is the use of commercial cartridges, which ensures reproducibility and standardization of the method for multiple analyses. An application of the SPE method to a survey of volatile NAm content in sausages and dried milk powder revealed no contamination (<0.3 ppb) of either of these food samples.

Keywords: *Volatile N-nitrosamines; GC/TEA; rapid solid phase extraction; sausage; milk powder*

INTRODUCTION

Many volatile NAm (Figure 1) are potent carcinogens in animal species including higher primates (Tricker and Preussmann, 1991). NAm require metabolic activation by cytochrome P450 for their carcinogenicity (Shu and Hollenberg, 1996), yielding an alkylating agent that can react with DNA and protein (Shuker and Bartsch, 1994). The presence of *N*-nitroso compounds in foods (Walker, 1990) is regarded as an etiological risk factor for certain types of human cancers (see IARC, 1993; Mirvish, 1975, 1995). Thus, there has been much interest to detect this class of carcinogenic compounds in foods and beverages, which are among the major source of human exposure to these compounds (Lijinsky, 1990). Carcinogenic NAm are formed by chemical reaction between nitrosatable secondary amines such as the naturally occurring dimethylamine in fish products, nitrosating agents formed from nitrate or nitrite which are used as preservatives (Cassens, 1995; Gangoli et al., 1994), or nitrogen oxides from combustion gases in direct heating or drying food processes.

The development of methodologies and instrumentation to detect volatile NAm over the last two decades has revealed contamination of NAm in numerous foods and beverages including cured meats and beers at the low ppb levels [for a recent review see Biaudet et al. (1996)]. Gas chromatography (GC) coupled to the specific thermal energy analyzer detector (TEA) is the most suitable and widely used analytical method to detect volatile NAm. The high specificity and sensitivity associated with TEA enables less than 50 pg of NAm to be detected following GC separation. Because of the selectivity of the TEA detector, the clean-up procedures may be considerably reduced (Fine and Rounbehler, 1975). Hence, the method used to extract, purify, and concentrate the volatile NAm from a food matrix is the limiting step in the analysis. The most commonly used method is based on a vacuum distillation prior to liquid−liquid extraction and concentration step (Mavelle et al., 1991). In this procedure, artifactual formation of NAm during the distillation step, where both nitrite in the cured meat product and amines are present, must be strictly controlled (Sen et al., 1987). This method is tedious and both time and solvent consuming. The method also requires a substantial amount of laboratory space for large equipment and distillation apparatus. Consequently, the method is not convenient for multiple analyses. Furthermore, the analysis of the least volatile NAm is not easy by the distillation technique. Pensabene and co-workers developed novel extraction methods including SPE (Pensabene et al., 1992; Pensabene and Fiddler, 1994) and more recently supercritical fluid extraction (Fiddler and Pensabene, 1996), in which the nitrosamines [*e.g.* *N*-nitrosodi-*n*-butylamine (NDBA) and *N*-nitrosodibenzylamine (NDBzA)] were detected. Their SPE method, however, still required large amounts of solvents (up to 300 mL of DCM, 145 mL of pentane, and 45 mL of ether) and did not use commercial cartridges, which is required for standardization. Therefore, we have developed a time- and solvent-sparing extraction method based on SPE. Our work focused on thermally processed sausages and is applicable for the analysis of a range of the most widely encountered volatile NAm, including the poorly volatile NAm NDBA, NDBzA, and *N*-nitroso-*N*-methylaniline (NMA) in various food products. Comparison of the data obtained by the SPE and distillation techniques is also presented. Finally, we report the results on the volatile NAm content of sausages, dried fish, and milk powder that were analyzed by the SPE method.

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S0021-8561(97)00232-X CCC: $14.00

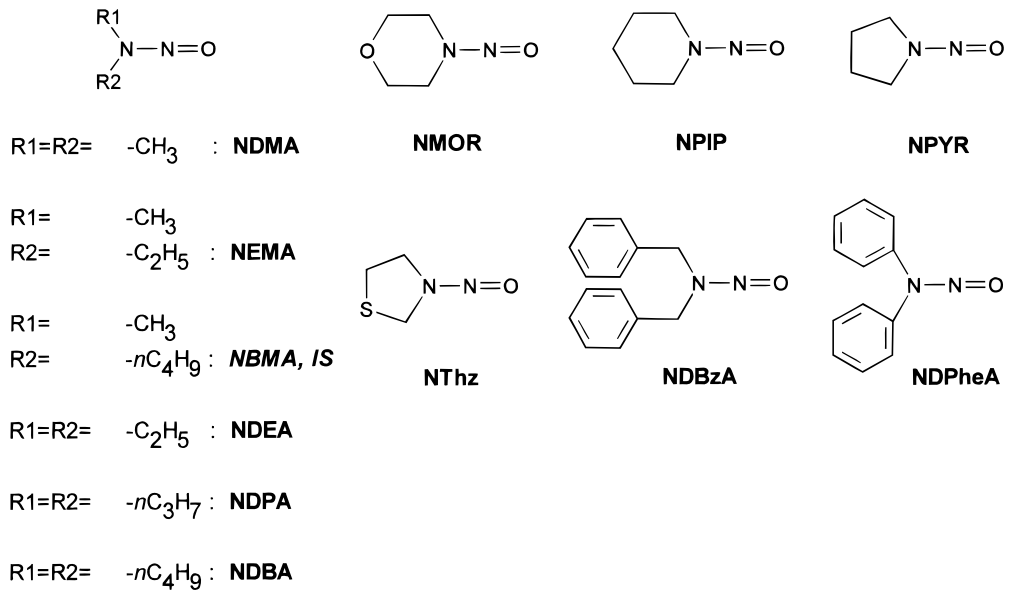

Figure 1. Chemical structures and abbreviations of *N*-nitrosamines (NAm).

MATERIALS AND METHODS

Caution! *Nitrosamines are potent, volatile carcinogens and must be handled carefully.*

Chemicals. Lichrosolv grade methanol, ethanol, DCM, and hexane, 0.1 N NaOH solution, high-performance TLC-Alufolien, Kieselgel 60F254 plates, and Extrelut 20 cartridges were purchased from Merck (Darmstadt, Germany). Chromabond Florisil (1 g) were obtained from Macherey-Nägel (Düren, Germany). All solvents and chemicals were checked to be free of nitrosamines. Some batches of Extrelut 20 revealed the presence of positive TEA response peaks on the chromatogram. Consequently, the Extrelut was prewashed with 60:40 (v:v) hexane−DCM (40 mL) and was allowed to dry overnight prior to utilization. Dibenzylamine 97%, morpholine 99+%, and thiazolidine 95% were obtained from Aldrich (Buchs, Switzerland). Radiolabeled *N*-nitrosodi[1-^{14}C]ethylamine ([^{14}C]NDEA) (26.8 mCi/mmol) was purchased from Chemsyn Science Laboratories (Lenexa, KS). The radiochemical purity was 98.8% on the basis of TLC analysis [R_f = 0.73; hexane−ether−DCM (5:7:10) (Leslie Gunatilaka, 1976)] using a Packard Instant Imager Analyzer.

The standards *N*-nitroso-*n*-butylmethylamine (NBMA, internal standard), and *N*-nitrosodiphenylamine (NDPheA) (Sigma, Buchs, Switzerland) were diluted to 10 μg/μL in ethanol. The internal standard, NBMA (500 pg/μL), was prepared by dilution of the stock solution in ethanol and aliquoted in an Eppendorf tube for a single application. Two Environmental Protection Agency (EPA) nitrosamine mixtures in methanol containing *N*-nitrosodimethylamine (NDMA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosodiphenylamine (NDPheA), and *N*-nitrosodi-*n*-butylamine (NDBA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosoethylmethylamine (NEMA), *N*-nitrosomorpholine (NMOR), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosopiperidine (NPIP) were obtained from Supelco (Buchs, Switzerland). They were combined to obtain a final concentration in methanol of 1 μg/μL of each NAm. Diluted solutions were prepared in ethanol at 100 and 1 ng/μL. The solutions were stored at −40 °C in the dark and were stable for at least 6 months. *N*-Nitrosothiazolidine (NThz) and NDBzA were prepared by nitrosation of their corresponding secondary amines as described by Pensabene et al. (1972). GC/MS analysis of the diluted ethereal extracts confirmed the identities of the respective NAm, which were 99% pure.

Test Foods. Sausages, dried fish powder, and dried milk powder were obtained from local markets. All the sausages contained nitrite which was labeled as a preservative. The sausages were grilled for 10−15 min at 240−260 °C in a restaurant kitchen and were stored in the dark at −40 °C until analysis.

Preliminary recovery experiments using [^{14}C]NDEA. Recovery experiments were done with 3 g of sausage spiked with 48.9 nCi of [^{14}C]NDEA (186 ng, 62 ppb) and homogenized in 3 mL of 0.1 N NaOH. Extrelut packing material (*ca.* 3 g) was added until complete absorption of liquid was achieved. The fluffy mixture was then packed into an empty Extrelut column. When required, a Florisil cartridge, pre-equilibrated with hexane, was placed in series after the Extrelut column. Elution was performed with the desired solvent, and the recovered fractions were weighed. [^{14}C]NDEA-containing fractions were quantitated by liquid scintillation counting with an LKB-Wallac 1219 Rackbeta counter.

Extraction and Concentration of NAm. A food sample (6.0 ± 0.1 g) mixed with 6 mL of 0.1 N NaOH and 200 μL of IS (0.5 ng/μL, 16.7 ppb) was homogenized for 3 min in a 50 mL glass beaker using a Polytron mixer (Kinematica GmbH, Luzern, Switzerland). Extrelut packing material was then added (6 g) and thoroughly mixed until complete absorption of the homogenate was achieved. The mixture was then transferred into an Extrelut 20 polypropylene column as described above.

The NAm were eluted from the Extrelut column with 40 mL of 60:40 (v:v) hexane/DCM, and the eluent was directly collected into a 25 mL Kuderna−Danish (K−D) receiving vessel equipped with a 40 mL flask (Supelco, Buchs, Switzerland). Antibumping glass beads, which also enhanced the evaporation rate, were added and a three-ball Snyder column was attached. The K−D apparatus was then placed in a water bath at 51 ± 1 °C (Tectron AG, Wald, Switzerland). The temperature was optimized to allow selective evaporation of the DCM but not of NAM in the minimum period. After 3−4 h, all the DCM was removed from the eluate mixture while the less volatile hexane remained.

The remaining hexane extract was then applied to a 1 g Florisil cartridge previously conditioned in 100% hexane (6 mL). Purification with Florisil gave the highest recoveries of NAm compared to other cartridges that were investigated, such as SiOH, NH_2, CN, diol, anionic, or cationic. Depending on the food matrix, a moderate pressure provided by a syringe was sometimes necessary to force the sample through the cartridge with a flow rate of *ca.* 1 drop/s. Subsequent washing of the cartridge with 8 mL of 100% hexane ensured that fat was removed from the cartridge. The Florisil cartridge was partially dried with 10 mL of air using a syringe. NAm were then eluted with 6 mL 95:5 (v:v) DCM/methanol into a 2 mL K−D vessel attached to a 40 mL flask and a three-ball Snyder column containing antibumping granules. Concentration to 0.5 mL took place rapidly in the water bath at 48 °C (*ca.* 20 min). After being cooled, the 40 mL flask was removed, several new antibumping granules were added, and the concentration

was extended to 0.2−0.3 mL final volume which was transferred into GC injector vials. Extended evaporation to near dryness drastically decreased the recovery of NDMA and to a lesser extent NEMA. Since the recovery for each NAm was different, the calibration was obtained by spiking a sausage with increasing amounts of the nine NAm, the internal standard (IS) NBMA being set at 16.7 ppb. Extractions were performed in duplicate, and each sample was injected three times into the GC/TEA.

Apparatus. GC/TEA analyses were performed with a Varian 3400 GC instrument (Palo Alto, CA) with a 1075 split/splitless capillary injector equipped with a splitless straight tube liner and a 8200 autosampler. The detector was a TEA model 502 B analyzer (Thermedics Detection, Chelmsford, MA). The TEA operating conditions were as follows: transfer line, 280 °C; furnace temperature, 500 °C; oxygen flow rate, 15 mL/min; vacuum pressure in the reaction chamber, 0.3 mmHg. A CTR Gas Stream Filter (Thermedics Detection) replaced the original liquid nitrogen cold trap. Changes in the TEA parameters such as oxygen flow rate and pyrolyzer and transfer line temperatures had no noticeable effects on the response.

The capillary column was an Optima 1701 (88% methyl, 7% phenyl, and 5% cyanopropyl) (25 m × 0.2 mm i.d.; film thickness, 0.25 μm) from Macherey-Nägel (Düren, Germany) and was connected to a fused silica capillary precolumn, 1 m × 0.53 mm i.d. The injection (injection volume 5 μL) was done in the splitless mode at 220 °C, turning to split mode after 1 min. The flow rate of helium carrier gas was adjusted to a head pressure of 23 psi, corresponding to a linear velocity of 35 cm/s at 50 °C. The oven program was as follows (for samples presenting interfering peaks eluting with NDBA, the GC program was modified as indicated in parentheses): 50 °C for 1 min then raise to 95 °C at 30 °C/min (not changed), then to 115 °C (120 °C) at 3 °C/min (2 °C/min), then to 220 °C (200 °C) at 40 °C/min (20 °C/min), and hold at this final temperature for 1 min (not changed). The retention times of the volatile NAm, given in their elution order, are as follows: NDMA, 4.0 min; NEMA, 4.5 min; NBMA IS, 7.1 min; NDEA, 5.2 min; NDPA, 8.1 min; NMOR, 8.7 min; NPYR, 9.1 min; NPIP, 9.6 min; NThz, 10.3 min; NDBA, 11.2 min; and NDBzA, 14.9 min. For the analysis of NDBzA, a higher temperature for the GC (220−230 °C) must be set to elute this semivolatile compound.

Identification of the NAm was confirmed by GC/MS in electron impact mode (EI) with a Hewlett Packard 5890 series II chromatograph equipped with a 7673 GC/SFC injector and coupled via a transfer line at 280 °C to a 5972 series MS detector. The injector port was set to 220 °C; injection of 1 μL was done in the splitless mode, turning to split mode after 1 min. Helium carrier gas was set to 35 cm/s in a constant flow mode. The capillary column and precolumn were identical to that used for GC/TEA analysis. The oven program was set at 60 °C for 2 min and then raised to 220 °C at 10 °C/min and held at this final temperature for 2 min.

UV Irradiation. To confirm the presence of NAm in the food samples, the glass injector vial containing the extract was placed 3 cm below the model ENF-260C/F (Spectronics Corporation, Westbury, NY) bench UV lamp (39 W) and irradiated at 365 nm for increasing times. At 15 min intervals, the sample was analyzed by GC/TEA and the peak area of the internal standard NBMA was used to monitor the photodecomposition of the NAm in the extract. The reaction was complete within 1 h. A positive control was performed with a standard solution containing the NAm standards.

Vacuum Distillation. Analyses were performed as described by Mavelle et al. (1991). The method consisted of a vacuum distillation (<2 mmHg, 70 °C) of a 20 g minced food sample mixed with 10 mL of ammonium sulfamate 10% in 1 M phosphoric acid as nitrosation inhibitor, 16.7 ppb of *N*-nitrosodiisopropylamine (NDiPA) as IS, 30 mL of twice-distilled water, and 40 mL of paraffin oil. The aqueous distillate, collected in a liquid nitrogen bath, was acidified with 0.1 N HCl solution and then extracted three times with 30 mL DCM. The combined extracts were dried and concentrated on a Kuderna−Danish evaporator at 52 °C to 1 mL. For the analysis, 10 μL was manually injected onto a 10% Carbowax 20M on Chromosorb WAW 80−100 mesh packed gas chromatographic stainless steel column (4.6 m × 3.2 mm o.d.) interfaced to a TEA detector. Argon (30 mL/min) was used as carrier gas.

RESULTS

Recovery Experiments with Radiolabeled [^{14}C]-NDEA. The principle of the SPE method is based on the interactions between the analyte and the functional groups of the adsorbents as a function of solvent. Preliminary studies were conducted to determine the most suitable solid phase support and appropriate solvents to extract nitrosamines from sausage using radiolabeled [^{14}C]NDEA. This NAm was chosen as representative of the volatile NAm of interest that can be found in sausages, in terms of both polarity and volatility.

The [^{14}C]NDEA spiked into sausage (60 ppb) was mixed with the diatomaceous earth Extrelut in an alkaline medium. More than 90% of the [^{14}C]NDEA was recovered with hexane, and only several percent were recovered with additional DCM. In this first step, the nitrosamines were separated from the nitrite contained in the sample which was retained on the resin, reducing the potential for artifactual nitrosation. A second SPE step was required for further purification of the NAm. Florisil was used for this purpose because it effectively adsorbs nitrosamines but not lipids. [^{14}C]NDEA was not detected in the hexane eluent or after elution with addition of hexane. The retained [^{14}C]NDEA was quantitatively eluted from the Florisil with 3 bed volumes of 5% methanol in DCM. In conclusion, tandem Extrelut−Florisil SPE is an efficient means to isolate [^{14}C]NDEA from sausage. The recovery of [^{14}C]-NDEA was greater than 90%.

Since NDEA is one of the most volatile NAm, we conducted experiments to optimize the concentration step of the extract using the Kuderna−Danish (K−D). Evaporation of the DCM under a stream of nitrogen to near dryness resulted in complete loss of [^{14}C]NDEA. Utilization of a heated water bath considerably reduces the volatility of [^{14}C]NDEA. However, the temperature must not exceed 52 °C. After 6 h at 52 °C in the K−D evaporator, 91% of the solvent was evaporated while the recovery of [^{14}C]NDEA was >90%. A higher temperature (56 °C) did not further concentrate the sample within the same time but caused loss of [^{14}C]NDEA (<60% recovery). The presence of boiling chips in the receiving vessel of the K−D evaporator enhanced the concentration rate without affecting the loss of [^{14}C]-NDEA.

Optimization of the Extraction Method and Recovery of *N*-Nitrosamines. NBMA was chosen as the IS because of its intermediate polarity and volatility and because it has not been reported to occur in foods. For recovery determination of the NAm, NBMA was added before the injection and then served as an external standard. A mixture containing NDMA, NEMA, NDEA, NDPA, NMOR, NPYR, NPIP, NDBA, and ND-PheA (Figure 1) was used to spike the sausage prior to homogenization and mixed with Extrelut. Elution with hexane quantitatively recovered only the most apolar NAm, NDEA, NDPA, NPIP, and NDBA, and *ca.* 50% of NEMA, while NDMA, NEMA (50%), NMOR, and NPYR were retained on the column. DCM was necessary for the recovery of the latter nitrosamines. Quantitative elution was achieved with a 60:40 (v:v) hexane/DCM

Table 1. Recovery (%)[a] of Eight NAm Spiked at Two Levels in a Sausage Sample[b]

spiked at	NDMA	NEMA	NDEA	NDPA	NMOR	NPYR	NPIP	NDBA
1 ppb	40 ± 7	60 ± 10	63 ± 6	67 ± 16	92 ± 12	89 ± 7	99 ± 6	nd[c]
10 ppb	58 ± 11	65 ± 7	70 ± 6	79 ± 8	92 ± 8	94 ± 9	99 ± 10	58 ± 13

[a] Mean and standard deviation of three independent analyses, each injected in triplicate. [b] NBMA (8.3 ppb) was added at the end of the procedure and was used as external standard. [c] nd, not determined because of the presence of some interfering peaks in the sample. NDPheA is not reported because its thermal decomposition within the GC did not allow its quantification.

mixture, where the minimum volume required to quantitatively elute the nine nitrosamines plus the IS from Extrelut was 40 mL. Whereas most of the NAm were eluted within the first 25 mL, an additional 15 mL of solvent was required to elute NDMA, NMOR, and NPYR.

The extract (40% DCM in hexane) contained large amounts of impurities, most of them being fats. Further clean-up of the extract was necessary before GC injection to avoid saturation and performance degradation of the capillary column. Thus the extract was applied to a Florisil cartridge after selective removal of DCM. A subsequent washing step with hexane removed most of the lipophilic compounds. However, there was some loss of the most nonpolar NAm, NDBA (*ca*. 25%), which was also partially eluted with hexane. Quantitative elution of the polar NAm from the Florisil cartridge was achieved with 95:5 (v:v) DCM/MeOH since neat DCM was not sufficiently polar to elute the remaining NAm. The method was shown to be free of artifactual NAm formation. When 50 ppm of morpholine, a rapidly nitrosated amine (Mirvish, 1975), was used to spike the sausage, no NMOR was detected, with a detection limit of <0.3 ppb.

The recoveries of eight NAm were measured at spikes of 10 and 1 ppb, in triplicate (Table 1). The recoveries of NAm were similar except for NDMA, for which a slightly lower recovery was obtained at 1 ppb. The differences in recovery observed between each NAm are explained by differing polarities which affected their elution from the columns. Some of the loss of NDMA, the most volatile nitrsoamine, occurs during the final concentration step where the solvent is reduced to 0.2−0.3 mL to achieve a detection limit of 0.3 ppb. The recovery of the IS NBMA was 67% ± 4% and is within the range of the other NAm. The coefficient of variation was 13.5% (6.0−23.9%) and 12.4% (8.6−22.4%) on average for 1 and 10 ppb, respectively. The SPE method can also extract NThz and NDBzA from sausage with comparable recovery when spiked at 10 ppb (S. Raoul, unpublished observations).

Recovery experiments for sausages spiked at 0.5, 2, and 7 ppb with the mixture of nine NAm were done in duplicate by two different investigators to determine intralaboratory reproducibility. Each sample was injected three times. No significant differences (<18%) in recoveries, with the exception of NDBA (up to 30% difference), were observed between the two operators.

GC/TEA Optimization. Although a packed column has been widely used for such applications (Biaudet et al., 1996), a capillary column with intermediate polarity was successfully employed. It provided superior separation and the required sensitivity with reduced analysis time (12 min) over packed columns in which the analyses lasted for 30 min. Although the inner diameter of the column was 0.2 mm, a 5 μL injection volume in the splitless mode was possible without affecting column performance. The use of a deactivated fused silica precolumn, which was periodically changed, prevented premature column deterioration. Such a large injection volume also increased the sensitivity to sub-ppb level. The use of capillary columns is also advantageous because of improved, narrow peak shape and resolution, which increased the confidence of analyte identity.

Optimization of the GC separation and TEA detection was performed with the standard reference solution containing the nine NAm and their identities were confirmed by GC/MS as described in Materials and Methods. The order of nitrosamine elution was the same in both systems, with the exception of DPheA, the latest eluting peak in the GC/MS chromatogram (data not shown). The MS of this peak showed a major ion at *m*/*z* 169 which is characteristic of diphenylamine (DPheA) as well as NDPheA (Pensabene et al., 1972). This peak was identified as diphenylamine by co-elution with the authentic amine. An on-column vs splitless injection showed similar GC profile and identical mass spectra. Thus, NDPheA, which is thermolabile (Welzel, 1971), appears to decompose within the capillary column or in the injector port and cannot be analyzed by GC under these conditions. The thermal decomposition of NDPheA also resulted in a positive response corresponding to $NO^{\bullet}$ at the solvent front, which is not observed when solvent is injected alone. This agrees with observations by Fine and Rounbehler (1975). The other nitrosamines were identified by comparison of their MS with those of the database. All spectra showed the characteristically important ions *m*/*z* 30 ($[NO]^+$) and 42 ($[CH_2{=}N{=}CH_2]^+$) and relatively large parent ions ($[M]^{\bullet+}$) (Pensabene et al., 1972).

Calibration. A five-level calibration curve (1, 2, 5, 10, and 20 ppb) was obtained for each NAm by spiking experiments in an uncontaminated sausage (Figure 2). A linear relationship between the area ratio vs concentration was obtained for all the volatile NAm. The statistical analysis indicated a detection limit at 0.3 ppb for NDMA, NEMA, NDEA, NDPA, NMOR, NPYR, and NPIP and of 1.7 ppb for NDBA. The higher value for NDBA is accounted for by a 5-fold lower slope of the calibration curve with respect to the other NAM. Under *ca*. 10 ppb, the variation is mainly driven by the response of the TEA signal to noise ratio. The confidence interval, covering the probability of a 95% estimate value obtained with the calibration curve, is about ±0.3 ppb for values <1 ppb and ±30% for values >1 ppb for all the NAm except NDBA (±60%).

Comparison of the SPE and Vacuum Distillation Techniques. To validate our approach, we compared the results of volatile NAm analysis for a thermally processed sausage obtained by the SPE and the distillation techniques. Because the NAm content of the analyzed sausage was <1 ppb (*vide infra*), we spiked the sausage with 2 and 10 ppb of the nine volatile NAm. The two methods were also used to analyze dried fish powder (Figure 3). The results (Table 2) obtained by the two methods are similar and close to the expected values. The differences observed for the least volatile NAm, NMOR, NPYR, and NDBA are explained by their

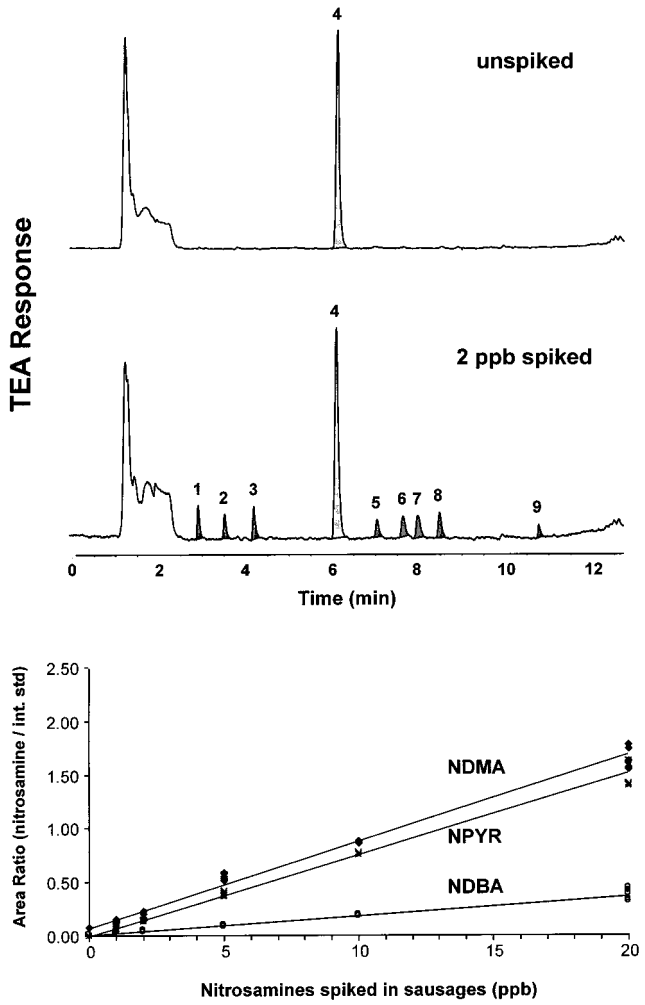

Figure 2. GC/TEA of sausage extract spiked with 0 and 2 ppb of volatile NAm. Peak identities: 1, NDMA; 2, NEMA; 3, NDEA; 4, NBMA (IS); 5, NDPA; 6, NMOR; 7, NPYR; 8, NPIP; and 9, NDBA. The IS, NBMA (peak 4), was added at 16.7 ppb. Calibration curve of three representative NAm spiked in sausage at the 0, 1, 2, 5, 10, and 20 ppb level.

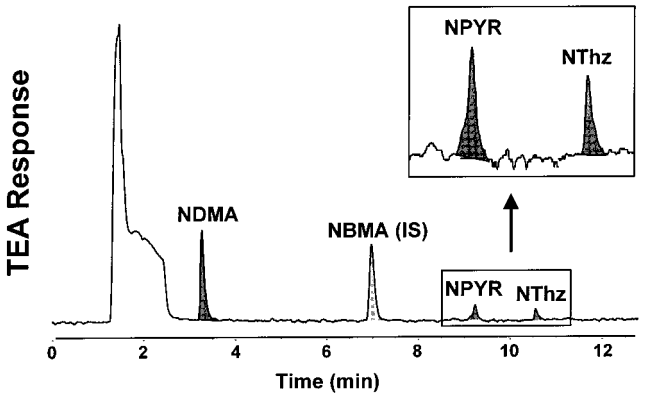

Figure 3. GC/TEA of dried fish powder extract [16.7 ppb NBMA (IS)]. Found NDMA at 12.5 ppb; NPYR, 1.4 ppb; NThz was not quantified.

poor recoveries by the vacuum distillation technique (Eisenbrand et al., 1983).

Survey of *N*-Nitrosamine Content in Sausages and Dried Milk Powder. Commercial smoked sausages are made from pork, beef, turkey, or mixtures. Nitrites and nitrates, as well as ascorbic acid, are often added. Most of the sausages are thermally processed during production, and the refrigerated product sold to the consumer is already cooked and ready to eat. The sausages may then be cooked again to desirable taste. NAm analysis of sausages was performed on commercial sausages and the same sausages after cooking (Table 3). NDMA, NDEA, NMOR, and NPIP were detected in several sausages. However, the quantity did not exceed 1 ppb, and most values were close to the detection limit. Cooking produced no differences. Several sausage extracts showed four TEA-positive peaks eluting after 10 min and one peak eluted at the same retention time as NDBA (Figure 4, bottom). Modification of the chromatographic conditions by changing the GC temperature program resulted in complete separation of spiked NDBA from the interfering peaks (Figure 4). The first peak was identified as NThz, *ca.* 0.5−1 ppb, by coelution with authentic chemical and UV-A experiments (*vide infra*). The identity of a large peak (*ca.* 20 ppb based on the TEA response) eluting at 10.5 min is not known. Attempts to identify this compound by coinjection with several other known NAm was unsuccessful. Co-injection of *N*-nitroso-*N*-methylaniline (NMA), a potential contaminant (Sen et al., 1990a), eluted as a broad peak at 10.7 min whereas the unidentified peak remained sharp at 10.5 min. The two peaks that eluted at 11.0 and 11.2 min are not *N*-nitroso compounds because exposure of the extract to UV-A light (Doerr and Fiddler, 1977) did not destroy them whereas the volatile NAm were quantitatively photodecomposed (Krull et al., 1979). The UV resistant nitramines, the oxidative products of NAm, which give a positive TEA response, are potential candidates (Walker and Castegnaro, 1980). Nitrodimethylamine was tested, but its retention time was 5.2 min. The C-nitroso and C-nitro compounds, which are also responsive to the TEA (Webb et al., 1983), are possible contaminants.

Five brands of commercial dried milk powder were analyzed for volatile NAm by the SPE method. We compared the results of duplicate analyses obtained for milk powder without spiking and with 2 ppb NAm spiking. No contamination (<0.3 ppb) of volatile NAm was observed for any of the five samples analyzed, while the NAm were readily detected in the spiked samples (Figure 5).

DISCUSSION

We have developed a rapid SPE method for the analysis of the most common volatile NAm which may be present in foods including sausages and dried foods such as fish or milk powder. For the latter, the vacuum distillation technique has not been successfully adapted (H. Biaudet, unpublished observation). Some SPE methods have been previously developed to extract volatile NAm as an alternative to the vacuum distillation technique and were shown to be free of artifactual formation (Pensabene et al., 1992). However, these surveys were restricted to a single matrix contaminated by one NAm (Havery et al., 1984; Sen et al., 1984, 1990b). More recently, Pensabene et al. (1994) developed an SPE method to analyze semivolatile NDBA and NDBzA in hams processed with elastic rubber netting. However, large volumes of solvent are required, typically 490 mL per analysis for 10 g of food sample. In our SPE method, both the amount of solvent (60 mL) and the amount of food sample required (6 g) are drastically reduced without affecting the sensitivity, which remains at the 0.3 ppb detection limit. The selective evaporation of DCM from the Extrelut eluent gave a reproducible final volume of hexane extract containing NAm and other co-eluting material, such as

Table 2. Analysis of Volatile NAm in Sausage (Unspiked, 2 ppb Spiked, and 10 ppb Spiked) and Dried Fish Powder: Comparison of the Results[a] (in ppb) Obtained by SPE[b] and Vaccum Distillation Techniques[c]

sample	method	NDMA	NEMA	NDEA	NDPA	NMOR	NPYR	NPIP	NDBA
dried fish	distillation	9.4, 10.2	nd[d]	nd	nd	nd	0.8, 0.8	nd	nd
	SPE	11.0, 12.2	nd	nd	nd	nd	1.4, 1.4	nd	nd
sausage									
unspiked	distillation	nd	nd	nd	nd	nd	nd	nd	nd
	SPE	nd	nd	nd	nd	nd	nd	nd	nd
2 ppb spiked	distillation	2.0, 2.1	1.9, 2.2	2.1, 2.3	1.7, 1.8	1.1, 1.6	1.6, 1.7	1.9, 1.9	0.9, 0.9
	SPE	1.9, 2.1	2.0, 2.1	2.1, 2.1	1.8, 1.9	1.9, 1.9	1.8, 2.0	1.9, 2.0	1.7, 1.8
10 ppb spiked	distillation	9.8, 10.2	10.0, 10.1	10.2, 10.2	8.4, 8.8	8.2, 9.2	8.0, 8.0	8.7, 9.4	2.6, 3.3
	SPE	10.4, 10.4	10.3, 10.5	9.5, 9.6	8.9, 9.7	9.8, 10.3	9.7, 10.4	9.3, 9.5	7.9, 8.4

[a] Values are from two independent extractions. [b] The detection limits of the SPE method are 0.3 ppb for NDMA, NEMA, NDEA, NDPA, NMOR, and NPYR and 1.7 ppb for NDBA. The estimates of nitrosamines are corrected for recovery by means of the internal standard. [c] The detection limits by the vacuum distillation technique are 0.15 ppb for NDMA and NEMA; 0.2 ppb for NDEA; 0.3 ppb for NDPA, NDBA, and NPIP; 0.5 ppb for NPYR; and 0.6 ppb for NMOR. [d] nd, not detected.

Table 3. Volatile Nitrosamine Analysis[a] (in ppb) of Uncooked and Cooked Sausages by the SPE Method

sausage no.	conditions	NDMA	NDEA	NMOR	NPIP
1[b]	uncooked	nd[c]	nd	nd	nd
	cooked	0.5, 0.3	nd	nd	0.4, 0.3
2[b]	uncooked	0.5, 0.4	0.6, 0.4	nd	1.1, 0.9
	cooked	nd	nd	nd	1.0, 0.8
3	uncooked	nd	nd	nd	nd
	cooked	nd	nd	nd	nd
4	uncooked	0.6	nd	nd	nd
	cooked	nd	nd	nd	nd
5[b]	uncooked	0.3	nd	0.4	nd
	cooked	0.4	nd	nd	nd
6	uncooked	nd	nd	nd	nd
7[b]	uncooked	nd	nd	nd	nd

[a] Only values for NAm above quantification limit are reported. They are from two independent extractions and are corrected for recoveries. [b] These extracts were analyzed with modified GC conditions due to the presence of NDBA-interfering peaks. [c] nd, not detected.

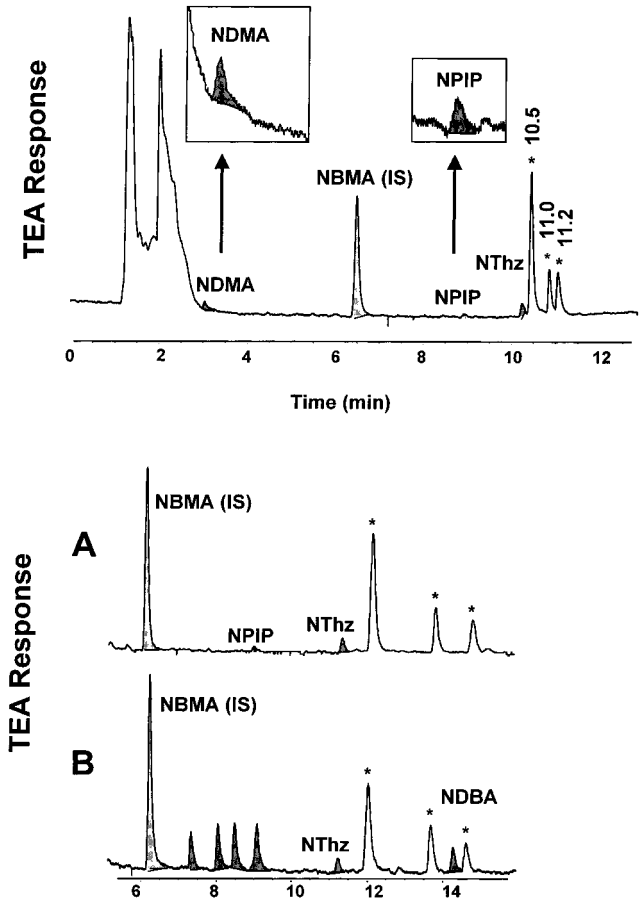

Figure 4. GC/TEA of sausage (no. 1, cooked). The upper chromatogram shows interfering TEA-positive peaks at 10.5, 11.0, and 11.2 min with 16.7 ppb NBMA (IS). The middle chromatogram (A) is the GC/TEA of sausage after modification of the temperature ramp of the GC oven. The lower chromatogram (B) is the modified GC/TEA of the sausage which was spiked with NAm standards at 3 ppb. Asterisks designate interfering TEA-positive peaks which are not NAm compounds.

lipids and pigments. Since the affinity of NAm for Florisil is influenced by interfering material in the extract, a reproducible SPE procedure with constant solvent volumes is essential.

Another advantage of our SPE method is the use of commercial cartridges which is necessary for standardization of the method. The reduced preparation and analysis time typically allows 10−12 simultaneous extractions to be performed per day compared to six extractions by many of the distillation techniques. The use of a capillary instead of a packed column also reduced the analysis time and provided superior chromatographic properties. Thus, there is a greater degree in confidence in analyte detection. The SPE method also enabled us to analyze the least volatile nitrosamines NDBzA and NThz, which have been found in smoked meat and fish products (Sen et al., 1986). NDBzA is not amenable to analysis by the vacuum distillation method (Pensabene and Fiddler, 1994). In addition, the SPE method is more adapted to extract NMOR, NPYR, and NDBA, which have poor recoveries by the vacuum distillation technique.

The estimated levels of volatile NAm in commercial processed sausages are similar to those reported in the literature (Tricker et al., 1991; Mavelle et al., 1991). In some of our analyses, we observed three unidentified interfering peaks that were not NAm. These unknown peaks are suspected to be nitramines or C-nitro or C-nitroso compounds on the basis of their resistance to UV-A irradiation.

The SPE method was successfully applied to dried milk powder. Less than 0.3 ppb of each of the NAm was found in this limited survey (five brands). The absence of NAm is important in this product because it is consumed by young children, and NDMA has been previously reported to occur in some powders at trace levels (Sen et al., 1984).

In conclusion, eight volatile and semivolatile NAm in several food products can be analyzed by our rapid SPE method. More analyses can be performed per day compared to the vacuum distillation technique, and less laboratory space and equipment are required. The amount of food sample and solvents required are also reduced relative to the vacuum distillation method

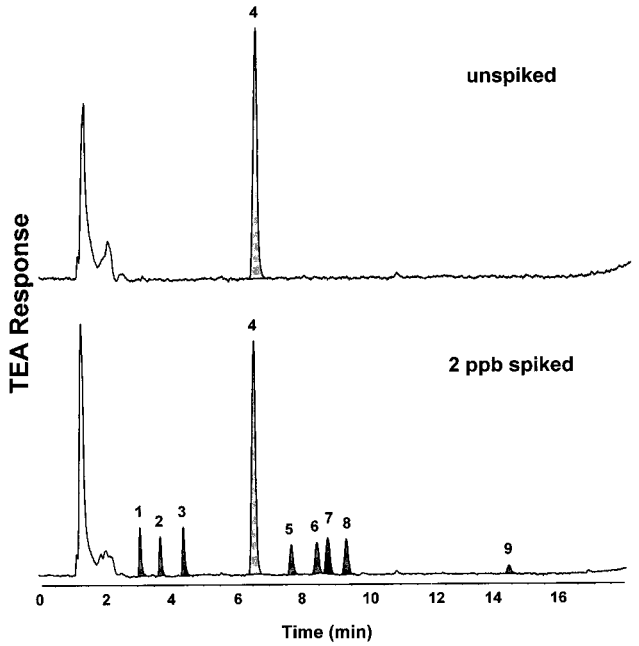

Figure 5. GC/TEA of dried milk powder. NBMA (IS) was spiked at 16.7 ppb. For peak identities see Figure 2.

without affecting the sensitivity, which was 0.3 ppb for all volatile NAm except NDBA (1.7 ppb).

ABBREVIATIONS USED

NAm, *N*-nitrosamines; NDMA, *N*-nitrosodimethylamine; NEMA, *N*-nitrosoethylmethylamine; NDEA, *N*-nitrosodiethylamine; NBMA, *N*-nitroso-*n*-butylmethylamine; NDPA, *N*-nitrosodi-*n*-propylamine; NDiPA, *N*-nitrosodiisopropylamine; NMOR, *N*-nitrosomorpholine; NPYR, *N*-nitrosopyrrolidine; NPIP, *N*-nitrosopiperidine; NDBA, *N*-nitrosodi-*n*-butylamine; NBPA, *N*-nitroso-*n*-butyl-*n*-propylamine; NDPheA, *N*-nitrosodiphenylamine; NDBzA, *N*-nitrosodibenzylamine; NThz, *N*-nitrosothiazolidine; NMA, *N*-nitroso-*N*-methylaniline; DPheA, diphenylamine; DCM, dichloromethane; GC/TEA, gas chromatography coupled to thermal energy analyzer; GC/EI-MS, gas chromatography coupled to mass spectrometer in electron impact mode; K−D, Kuderna−Danish evaporator; SPE, solid phase extraction; TLC, thin-layer chromatography; ppb, parts per billion (ng/g or μg/kg); MS, mass spectrum; IS, internal standard.

ACKNOWLEDGMENT

We thank J.-M. Aeschlimann (Nestec Ltd., Lausanne, Switzerland) for the statistical analysis.

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Received for review March 21, 1997. Revised manuscript received September 15, 1997. Accepted September 17, 1997.[⊗]

JF970232Q

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1997.