Simultaneous Determination of 2-(Hydroxymethyl)-*N*-nitrosothiazolidine-4-carboxylic Acid and 2-(Hydroxymethyl)-*N*-nitrosothiazolidine in Smoked Meats and Cheese

Nrisinha P. Sen,* Philander A. Baddoo, Stephen W. Seaman, and Dorcas Weber

Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada K1A 0L2

A method is described for the determination of the two title compounds in various cured smoked meats, including bacon, smoked poultry products, and smoked cheeses. It is based on (a) extraction of the sample with methanol or acetonitrile, (b) removal of fats and lipids by partitioning of the extract with isooctane, (c) cleanup and separation of the two compounds on an acidic alumina sample preparation cartridge, (d) derivatization of 2-(hydroxymethyl)-*N*-nitrosothiazolidine-4-carboxylic acid to its methyl ester (HMNTCA-ME), and (e) determination of HMNTCA-ME and 2-(hydroxymethyl)-*N*-nitrosothiazolidine (HMNTZ) by high-performance liquid chromatography thermal energy analyzer technique. The recoveries of HMNTHZ and HMNTCA added to various products at 10–20 and 50–200 ppb, respectively, varied between 82–112% (mean, 94%) and 58–101% (mean, 79%). Confirmation was carried out by gas–liquid chromatographic–mass spectrometric analysis of HMNTHZ as its *O*-methyl ether and of HMNTCA-ME as its heptafluorobutyryl derivative.

INTRODUCTION

Recent research has shown that certain foods, especially cured meats and fried bacon, contain a number of both volatile and nonvolatile *N*-nitroso compounds (Hotchkiss, 1987; Sen, 1990; Tricker et al., 1984). 2-(Hydroxymethyl)-*N*-nitrosothiazolidine-4-carboxylic acid (HMNTCA) and 2-(hydroxymethyl)-*N*-nitrosothiazolidine (HMNTHZ) are two of the latest nonvolatile *N*-nitroso compounds that have received considerable attention. HMNTCA was first detected in raw bacon by Massey et al. (1985) and later also reported to be present at 10–1300 ppb levels in various smoked meats and cheeses (Mandagere, 1986; Tricker and Kubacki, 1991; Sen et al., 1991a). HMNTHZ (traces–13 ppb) has been detected mainly in fried but not in raw bacon or in any other cured smoked meats (Sen et al., 1989). This suggests that it is most likely formed by heat-induced decarboxylation of HMNTCA during frying of bacon (Figure 1). Nothing is known about the carcinogenicity of the two compounds, but HMNTHZ is mutagenic (Umano et al., 1984). Because of this, there is considerable interest as to their presence in foods.

A variety of methods have been reported for the determination of both volatile and nonvolatile *N*-nitroso compounds in foods [reviewed by Massey (1988) and Sen and Kubacki (1987)]. In their preliminary study, Massey et al. (1985) reported a brief description of the method used to isolate and detect HMNTCA in raw bacon. The quantitation was carried out by high-performance liquid chromatography thermal energy analyzer (HPLC-TEA) determination of the underivatized HMNTCA. On the other hand, Mandagere (1986) first derivatized it to its methyl ester before analyzing by gas–liquid chromatography TEA (GLC-TEA). No details of the various steps or of other analytical parameters (e.g., percent recovery, precision) of the method were reported. In our preliminary work, we observed that although these methods were workable, they required further refinement and modification. Furthermore, there was a need for a method for the direct simultaneous determination of both HMNTCA and HMNTHZ that would be useful for investigating heat-induced decarboxylation of HMNTCA to HMNTHZ. We have developed such a method, the details of which are reported in this paper.

Figure 1. Chemical structures of HMNTCA and HMNTHZ and conversion of the former to the latter.

MATERIALS AND METHODS

Apparatus. The HPLC-TEA unit used for the determination of HMNTCA and HMNTHZ was as described previously (Sen et al., 1989). A 250-mm × 4.1-mm (i.d.) stainless steel column packed with Lichrosorb Si 100 (5×10^{-6} m) (Alltech/Applied Science, State College, PA 16804) was used as the analytical column. Both isocratic programming (mobile phase, 20% acetone in *n*-hexane) and solvent programming (mobile phase, 5% acetone in *n*-hexane increased linearly to 20% over 10 min) using a mobile phase flow rate of 2 mL/min were used for the HPLC analysis. The TEA was operated as described previously (Fine et al., 1976). A mixture of dry ice and ethanol was used to cool the two cold traps.

A VG Analytical hybrid mass spectrometer (Model 7070 EQ) attached to a Varian (Model Vista 6000) gas–liquid chromatograph was used for GLC–MS analysis. Some samples (e.g., pure standards) were analyzed using a direct probe. A 30-m × 0.22-mm (i.d.) fused silica capillary column coated with DB-5 (0.25×10^{-6} m film thickness) (J&W Scientific Inc., Folsom, CA 95830) was used for the GLC separation. Temperature programming used was 70 °C for 1 min, raised to 150 °C at 50 °C/min and then to 220 °C at 3 °C/min.

Reagents. L-Cysteine and glycolaldehyde were purchased, respectively, from Aldrich Chemical Co. (Milwaukee, WI) and Sigma Chemical Co. (St. Louis, MO). All organic solvents used were of glass-distilled varieties. Acidic alumina for column chromatography was supplied by ICN Biomedicals, Inc., K & K Laboratories (Plainview, NY). All other reagents were of analytical grade. Acidic alumina Sep-Pak (2 mL) and reversed-phase LC-18 (3 mL) sample preparation cartridges were pur-

* To whom correspondence should be addressed.

0021-8561/92/1440-0221$03.00/0 Published 1992 by the American Chemical Society

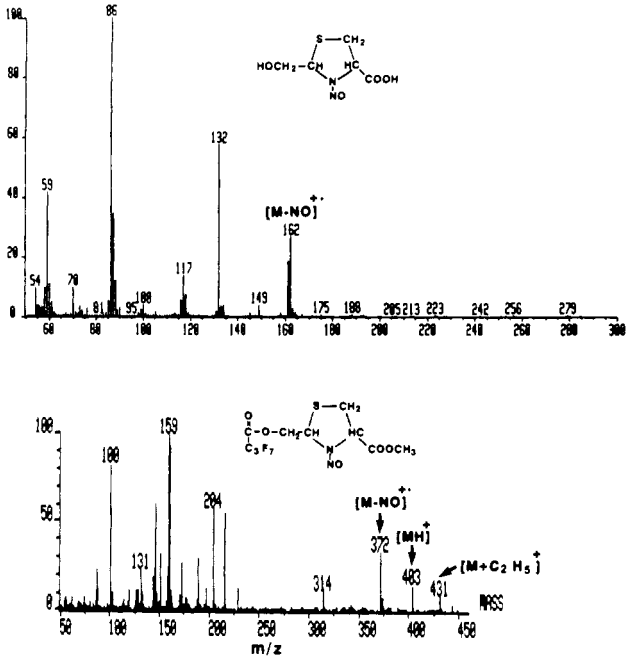

Figure 2. (Top) Electron impact ionization mass spectrum of HMNTCA standard (probe sample). (Bottom) Chemical ionization mass spectrum of HMNTCA methyl ester heptafluorobutyryl derivative using methane as the ionizing gas (sample introduced through GLC).

chased from Waters Associates (Milford, MA) and Supelco Canada Ltd. (Oakville, ON, Canada), respectively.

HMNTHZ and HMNTCA Standards. HMNTHZ was synthesized as described previously (Sen et al., 1989). HMNTCA was prepared by nitrosation of 2-(hydroxymethyl)thiazolidine-4-carboxylic acid (HMTCA), which was synthesized by condensation of L-cysteine and glycolaldehyde according to the method of Umano et al. (1984). The only modification made was that the reaction mixture was heated in a boiling water bath in a nitrogen atmosphere (to prevent oxidation of the SH group) and a reflux condenser was attached to the flask to prevent concentration during heating. The product (HMTCA) was crystallized from a mixture of ethanol and water (1:1), washed with ice-cold ethanol, and air-dried before use; its identity was confirmed by mass spectrometry (not shown).

About 55 mg of HMTCA was dissolved in 20 mL of water and then mixed with 150 mg of sodium nitrite. The pH of the solution was adjusted to ~2.5 (±0.2) with 1 N H_2SO_4, and the mixture was allowed to stand at room temperature for 2 h in the dark. At the end of this period, excess nitrite was destroyed by the addition of 300 mg of sulfamic acid to the solution, requiring 10 min of reaction time. The solution was saturated with sodium sulfate and extracted with three 30-mL portions of ethyl acetate, and the ethyl acetate extract was dried for 2 h over anhydrous granular sodium sulfate. The extract was then filtered, the filtrate concentrated to a small volume (~2 mL) using a rotary evaporator (under vacuum), and the residue transferred with rinsing to a small screw-cap vial. Finally, the solution was evaporated to near dryness by blowing down with a gentle stream of nitrogen (water bath ~30 °C). HMNTCA was obtained as a light yellow oil which on mass spectrometry (direct probe) gave a spectrum (Figure 2) with major fragments at m/z 162, 132, 117, 86, and 59, corresponding to $(M-NO)^{\cdot+}$, $(M-NO-CH_2O)^{\cdot+}$, $(M-NO-COOH)^+$, $(C_3H_4NS)^+$, and $(C_2H_3S)^+$, respectively. The molecular ion at m/z 192 was not visible. However, when the compound was derivatized to its methyl ester heptafluorobutyryl derivative, as described later, and analyzed by GLC–MS using the chemical ionization (CI) mode, the protonated molecular ion was clearly visible as a fragment at m/z 403 $(MH)^+$ and an adduct ion at m/z 431 $(M + C_2H_5)^+$ (Figure 2).

An aliquot of the underivatized HMNTCA standard was further purified by chromatography on an acidic alumina column (HMNTCA finally eluted with 1 N H_2SO_4). The eluate was extracted with ethyl acetate and the extract dried and concentrated as above. On HPLC-TEA analysis (as methyl ester), both the unpurified and purified standards gave very similar (peak heights within ±5%) results, suggesting that the former could also be used as an analytical standard. Because of larger quantity of the unpurified standard available to us, it was used in most of our studies.

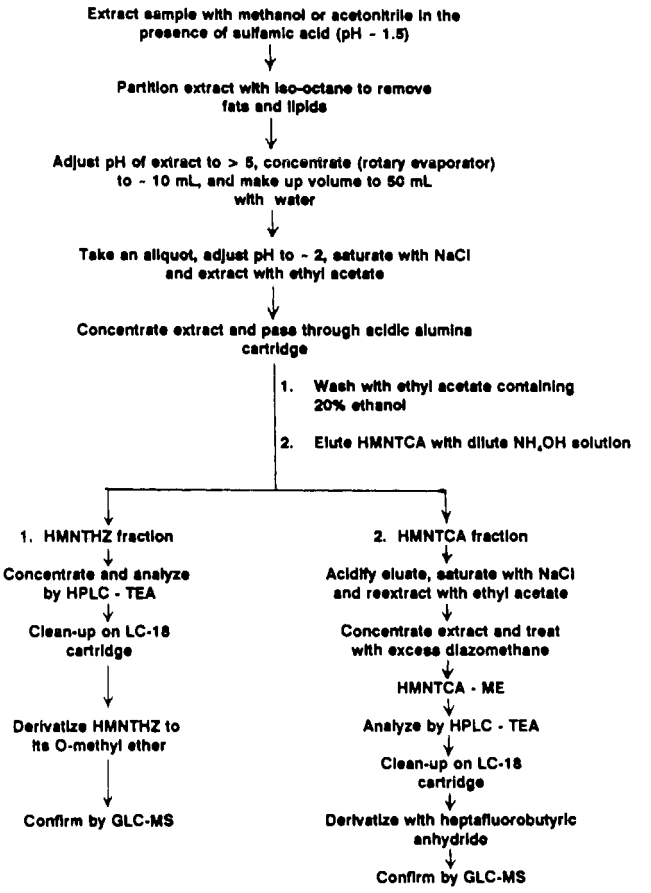

Figure 3. Flow diagram of the overall analytical scheme.

A 1–2-mg aliquot of each compound was accurately weighed on an ultramicro balance and dissolved in ethyl acetate to prepare the necessary standards. HPLC-TEA analysis of HMNTCA resulted in two peaks, presumably corresponding to the syn and anti isomers. Similar observations had also been made by Massey et al. (1985). HMNTHZ, on the other hand, gave only one peak on HPLC-TEA analysis (see later).

Caution. Since most *N*-nitroso compounds are potent carcinogens (Preussman and Stewart, 1984), proper precautions should be taken while handling or working with these compounds.

Procedure. An outline of the overall method is presented in the form of a flow diagram (Figure 3). The details are as follows.

(*a*) *Extraction.* A 10–20-g aliquot of a well-homogenized sample was mixed with 10 mL of 0.5 N H_2SO_4 solution containing 1% dissolved sulfamic acid (to prevent artifactual formation), and the mixture was extracted with 100 mL of acetonitrile (for processed meats only) or methanol (for all other products including bacon) for 2 min using a Sorval Omni Mixer (Du Pont Instruments, Newton, CT). After filtration (Büchner funnel), the residue was extracted further with two 60-mL portions of the appropriate solvent and then filtered as above. The entire contents in the mixer were poured on the filter funnel and rinsed with an additional 30 mL of solvent. The combined filtrate and the washings were transferred to a separatory funnel and extracted with 80 mL of isooctane, which was discarded.

One milliliter of NH_4OH solution (one part concentrated NH_4OH plus four parts water) was added to the extract and the mixture was evaporated to 10 mL using a rotary evaporator (water bath 35–40 °C; under subdued light). The residue was quantitatively transferred into a 50-mL glass-stoppered graduated cylinder with adequate rinsing of water. The volume was made up to 50 mL with water. A 10–25-mL aliquot was saturated with

sodium chloride, the pH of the solution adjusted to 2–2.3, and the solution extracted with three 50-mL portions of ethyl acetate. The combined extract was concentrated to ~1 mL by first evaporating using a rotary evaporator, transferring quantitatively to a test tube, and then blowing down with a gentle stream of nitrogen. Extracts were not dried over anhydrous sulfate at any stage of the procedure.

(b) *Separation of HMNTHZ from HMNTCA.* The acidic alumina cartridge was deactivated and conditioned by passing 3 mL of 20% water in acetonitrile, waiting for 30 min, and then washing with 5 mL of ethyl acetate. The concentrated sample extract from (a) above was passed through the cartridge, and the cartridge was washed with 5 mL of ethyl acetate containing 20% ethanol. The combined effluent and washings were collected in a graduated test tube, and the solution was concentrated to 1 mL under a gentle stream of nitrogen. This was the HMNTHZ fraction. Finally, HMNTCA was eluted from the cartridge with 4 mL of dilute NH_4OH solution (one part concentrated NH_4OH plus four parts water) and the eluate collected in a rotary evaporation flask (100 mL). The eluate was evaporated to ~2 mL (rotary evaporator), acidified to pH 2–2.5 with 1 N H_2SO_4 (~4 mL), and saturated with sodium chloride. The solution was extracted with ethyl acetate and the extract concentrated (without drying) to 1 mL as described above at the end of section a.

(c) *Esterification of HMNTCA.* The fraction containing HMNTCA was evaporated to ~0.1 mL under a gentle stream of nitrogen, and the residue was esterified by treating with excess diazomethane as described previously (Sen et al., 1983). An aliquot of standard HMNTCA (1–4 μg) was methylated in the same manner to yield HMNTCA methyl ester (HMNTCA-ME).

(d) *HPLC-TEA Analysis.* Prior to analysis, each of the above fractions was evaporated to ~0.1 mL under a stream of nitrogen and the residue was reconstituted with 0.9 mL of 20% acetone in *n*-hexane (the mobile phase). A 20–40-μL aliquot of each was analyzed by HPLC-TEA using either the isocratic (for both HMNTHZ and HMNTCA-ME) or the solvent programming mode (for HMNTCA only) as described under Apparatus.

(e) *GLC-MS Confirmation.* If a positive result was obtained for either HMNTHZ or HMNTCA-ME by HPLC-TEA, the respective fraction was further cleaned up and the compound was converted to an appropriate derivative at the OH position and then analyzed by GLC–MS as described below. This was, however, done only in a few selected cases just to demonstrate the applicability of the technique.

Each extract was again concentrated to ~0.1 mL under a stream of nitrogen and then mixed (vortex mixer) with 2 mL of water. The extract was cleaned up on a LC-18 cartridge as described previously (Sen et al., 1990, 1991b). Basically, the procedure involved washing the cartridge (after the sample was added) with 3-mL portions of water and 20% methanol in water and eluting HMNTHZ and HMNTCA-ME from the respective cartridges with 50% methanol in water and 75% methanol in water. The respective fractions were diluted with water and re-extracted with ethyl acetate. The extracts were dried over anhydrous sodium sulfate, and the dried extracts were filtered and concentrated to a small volume (~0.05 mL) in a graduated test tube as described above. HMNTCA-ME was derivatized to its heptafluorobutyryl (HFB) derivative and HMNTHZ to its *O*-methyl ether as described previously (Sen et al., 1978, 1989).

GLC–MS confirmation was carried out using the high-resolution (5–10K) selected ion monitoring (SIM) technique. The fragments monitored for the confirmation of HMNTHZ *O*-methyl ether were at *m/z* 162 $(M)^{\cdot+}$, 132 $(M-NO)^{\cdot+}$, and 117 $[M-(CH_2OCH_3)]^+$ and that for HMNTCA-ME-HFB derivative was at *m/z* 372 $(M-NO)^{\cdot+}$. Full-scan mass spectrum of HMNTCA-ME-HFB was also recorded for the isolated HMNTCA in one sample.

RESULTS AND DISCUSSION

Although HMNTHZ can be analyzed directly by the HPLC-TEA (Sen et al., 1989), the technique proved to be unsuitable for the determination of underivatized HMNTCA. Because of its acidic nature, an aqueous or acidic organic mobile phase was required for chromatography. Aqueous mobile phases "poison" the TEA pyrolysis tube and cause erratic and declining responses. The presence of moisture also freezes up the TEA cold traps. This ruled out the use of a reversed-phase system with TEA detection. Also, in the normal-phase system (silica column) using acidified organic mobile phases (e.g., a mixture of acetone in *n*-hexane acidified with trifluoroacetic or acetic acids), there was unacceptable background noise because of thermal decomposition of the organic acids in the TEA furnace. In view of these difficulties, an attempt was made to try a newly developed HPLC post-column chemical denitrosation technique (Havery, 1990) which is compatible with reversed-phase chromatography and aqueous mobile phases. The technique worked well with HMNTCA standard, but HMNTCA resolved poorly from other *N*-nitrosamino acids (e.g., *N*-nitrosoproline and *N*-nitrosothiazolidine-4-carboxylic acid) which are often present in cured smoked meats. Hence, the technique was abandoned.

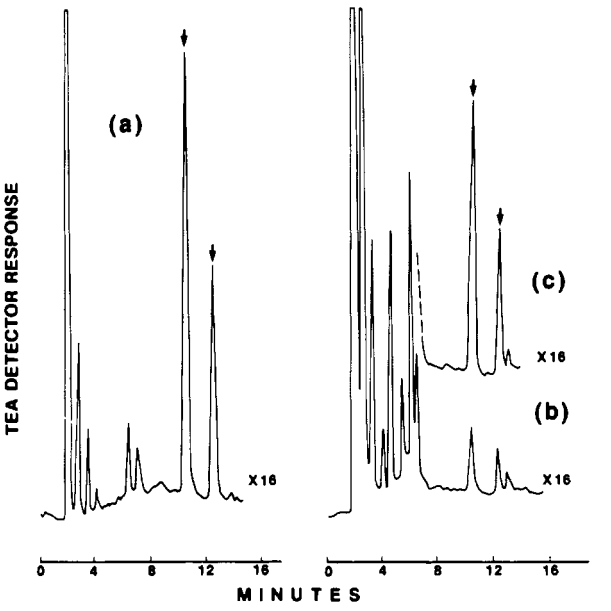

Figure 4. HPLC-TEA chromatograms: (a) 80 ng of HMNTCA standard (analyzed as HMNTCA-ME); (b) 20 μL/1 mL final extract of HMNTCA fraction (derivatized) from a raw bacon showing the presence of about 55 ppb of HMNTCA; (c) 20 μL/1 mL of the corresponding derivatized fraction from the same bacon after spiking 20 g of bacon with 4 μg of HMNTCA (200 ppb). Note: only half of the extract, which contained only 2 μg of spiked HMNTCA, was used for the analysis (98% recovery in this case). The peaks eluting before 8 min are also present in the blank; they originate from diazomethane.

To overcome the above difficulties, HMNTCA was esterified first with diazomethane before analysis by HPLC-TEA using organic mobile phases that are compatible with the TEA. This produced excellent chromatograms (Figures 4 and 5) and also resolved HMNTCA-ME from the methyl esters of *N*-nitrosoproline and *N*-nitrosothiazolidine-4-carboxylic acid, both of which eluted earlier (not shown). However, being similar in chemical structure, HMNTCA-ME did not resolve well from HMNTHZ. This was solved by first separating the two underivatized compounds on the acidic alumina cartridge, and then analyzing each fraction separately as outlined in the flow diagram (Figure 3).

The acidic alumina cartridge cleanup served two main purposes. Not only did it separate HMNTCA from HMNTHZ, it also resulted in considerable cleanup of the HMNTCA fraction. A better cleanup could be achieved, if desired, by judicious washings with increasingly polar solvents (e.g., ethanol or water) because HMNTCA could

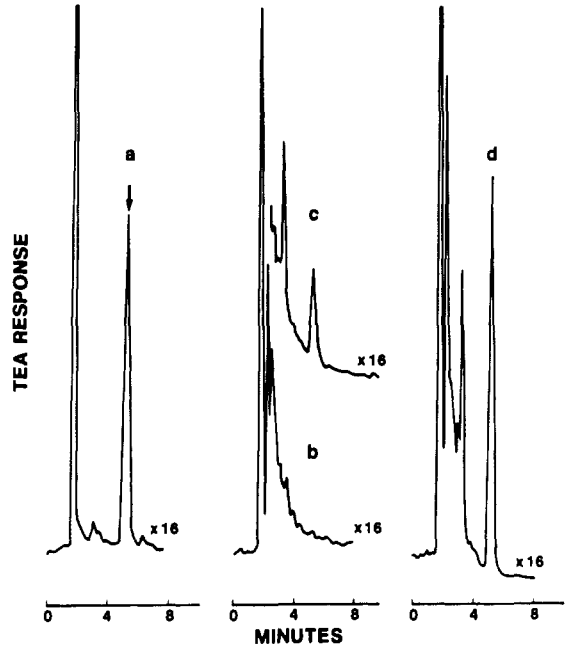

Figure 5. HPLC-TEA chromatograms from the analysis of underivatized HMNTHZ: (a) 16 ng of standard; (b) 20 μL/1 mL of extract of raw bacon; (c) 20 μL/1 mL of extract of the same bacon fried (control); (d) 20 μL/1 mL of extract of fried bacon obtained from HMNTCA-spiked (spiking level, 386 ppb) of the same raw bacon. Note increased formation of HMNTHZ due to heat-induced decarboxylation of the spiked HMNTCA.

Table I. Recovery of HMNTCA and HMNTHZ from Various Samples of Bacon, Smoked Meats, and Cheeses

	HMNTCA		HMNTHZ	
sample	spiking level, ppb	% recovery[a]	spiking level, ppb	% recovery[a]
frankfurter	200	76	20	102
smoked meat	100	99	10	90
	50	80	–[b]	–
	20	66	10	93
smoked cheese	200	78	–	–
	200	73	–	–
	200	70	20	91
smoked cheese	200	81	10	96
	200	64	10	82
raw bacon	200	98	–	–
fried bacon	200	101	10	85
raw bacon	200	87	20	112
raw bacon	200	58	20	94

[a] Most samples chosen for the recovery study were negative for these two compounds. In the other cases (e.g., raw bacon) where the sample contained some HMNTCA, its value was subtracted prior to calculation of the recovery data. [b] Not carried out.

be eluted only with a strong acid (e.g., 3 N H_2SO_4) or base (e.g., 1:4 NH_4OH). The latter was chosen because it consistently gave better recoveries than the former. Recovery of HMNTHZ through the cartridge was virtually quantitative, whereas there was some loss (5–10%) of HMNTCA. The recoveries of HMNTHZ added to various smoked meats, bacon, and cheeses ranged between 82% and 112% (average, 93.8%), and that for HMNTCA ranged between 58% and 101% (average, 79.3%) (Table I). The spiking levels chosen in these recovery studies reflect average levels of HMNTHZ and HMNTCA expected to be found in these products. The recovery values for HMNTCA obtained in this study are comparable or better than those reported for other hydroxylated *N*-nitrosamino acids (e.g., *N*-nitrosohydroxyproline) (Tricker et al., 1984). There appear to be no published data on the recovery values for HMNTCA from spiked smoked meats or cheeses. The minimum detection limit ($S/N \geq 2$) of the method was estimated to be approximately 2 ppb for HMNTHZ and 10 ppb for HMNTCA.

The precision of the method appeared to be highly satisfactory, especially if one considers its complexity. Replicate analyses in most cases gave results within ±20%. For example, duplicate analyses of fried bacon gave HMNTHZ values of 5.2 and 6.3 ppb, and quadruplicate analyses of a fried Polish coil sausage gave HMNTHZ values of 9.7, 8.9, 7.5, and 11.7 ppb. Similarly, replicate (n = 5) analyses of a smoked chicken sausage gave an average HMNTCA value of 32.1 ± 6.6 ppb, and duplicate analyses of a raw bacon gave HMNTCA values of 39 and 42.4 ppb.

There are four critical steps in the procedure that an analyst should be aware of while using the method. First, prior to concentration of the initial sample extract using the rotary evaporator, the pH of the extract must be brought up to 5–7 by adding dilute NH_4OH solution as described. Concentration at an acidic pH led to considerable losses of HMNTCA, presumably due to breakdown of the compound. Second, sample extracts in ethyl acetate must not be dried over anhydrous Na_2SO_4 or Drierite. This will lead to inconsistent and lower recoveries of HMNTCA. Third, if acetonitrile is used for extraction of the sample, it must be removed completely before treatment of the extract with diazomethane because acetonitrile was found to interfere with methylation. Finally, methylation of cheese extracts was found to consume excessive amounts of diazomethane (probably due to the presence of excess free fatty acids). Therefore, a smaller amount of final extract (equivalent to 5 g of cheese) should be used for this purpose. All of these steps are very critical for the determination of HMNTCA in foods but have not been pointed out or investigated previously. Also, it should be noted that of the two solvents used for extraction, methanol gives a smoother product with all samples and, therefore, results in a more efficient extraction, whereas acetonitrile does so only with well-comminuted products (e.g., processed meats) but results in a slightly cleaner initial extract. Hence, different solvents are used for extraction for different products.

The loss of HMNTCA during drying of ethyl acetate extracts was unexpected. Although the exact reason for this is unclear, it is possible that the drying agents or some unknown impurities present in them could have adsorbed the compound. Similar observations were made by Ho et al. (1981), who failed to recover *N*-nitrosodiethanolamine after it was dried over anhydrous calcium sulfate. Adsorption on the glass surface was thought to be the cause of the loss in that case. HMNTCA-ME could, however, be recovered in good yields (>90%) after drying over anhydrous sodium sulfate. This was necessary and helpful for derivatization using HFBA, a step where moisture can interfere with the reaction.

The method has been successfully used for the analysis of a variety of cured smoked meats, bacon (both raw and fried), smoked chicken and turkey, and smoked cheeses. It was also used to investigate the possibility of heat-induced decarboxylation of HMNTCA to HMNTHZ during frying of bacon. The details of these studies will be published elsewhere (Sen et al., 1991a). The data generated using our method clearly demonstrated that HMNTCA in raw bacon could decarboxylate to HMNTHZ

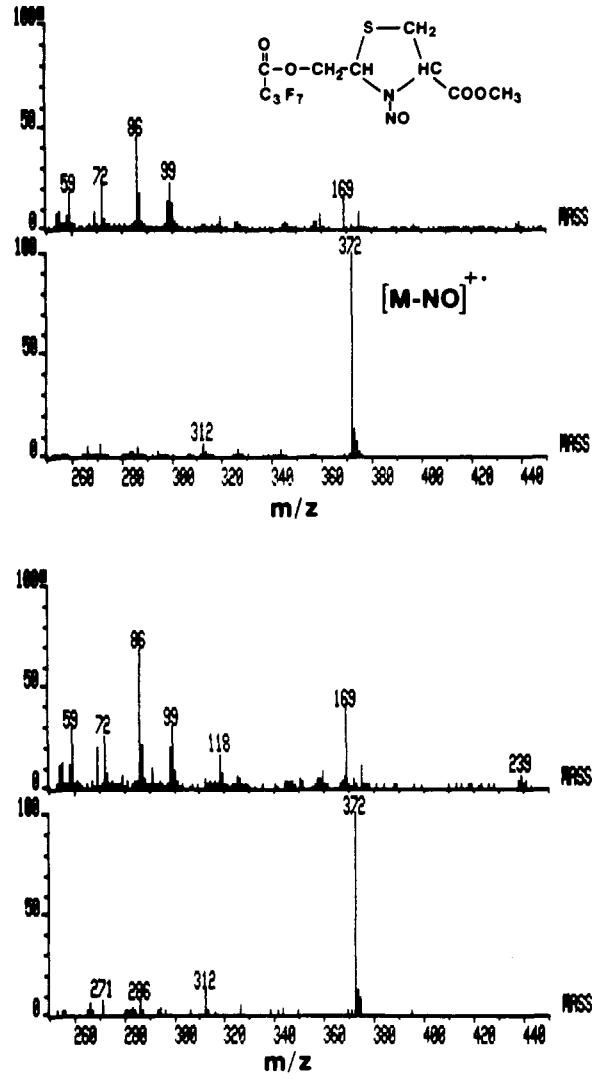

Figure 6. Electron impact ionization mass spectrum of standard HMNTCA-ME heptafluorobutyryl derivative (top) and that of HMNTCA isolated from a smoked raw bacon.

under frying conditions. When raw bacon, containing no HMNTHZ, was spiked with HMNTCA and then fried, the fried product contained significantly higher levels of HMNTHZ than the corresponding control fried bacon. Typical chromatograms obtained from such studies are shown in Figure 5.

Since the initial extraction of all samples was carried out at an acidic pH with added sulfamic acid, it is highly unlikely that the method is prone to artifactual formation of *N*-nitroso compounds. Sulfamic acid is well-known for its nitrite-scavenging property, and for this reason many researchers incorporate it in the extracting solvent to eliminate or minimize artifactual formation (Pensabene and Fiddler, 1985; Kubacki et al., 1984). This was further corroborated by analyzing a smoked ham, which was negative for HMNTCA, with 50 ppm levels of added HMTCA. No evidence of HMNTCA formation was observed. For a similar reason, it is also highly unlikely that the method is prone to artifactual formation of HMNTHZ.

The cleanup procedures outlined in the method under GLC–MS Confirmation seemed to be adequate. Thus far, we have successfully used the technique for the confirmation of HMNTHZ in two samples of fried bacon and one fried Polish coil sausage and of HMNTCA in two samples of fried bacon and one each of raw bacon and smoked frankfurter. The full mass spectrum of HMNTCA isolated from the raw bacon is shown as an example in Figure 6. The mass spectrum of the isolated compound (analyzed as HMNTCA-ME-HFBA derivative) and that of the standard are similar; both show a strong fragment at m/z 372 corresponding most likely to $(N\text{–}NO)^{\bullet+}$. As in the case of HPLC-TEA, the above derivative gave two peaks on GLC analysis. The major peak was used for GLC–MS confirmation. In addition, the identity of the compound was confirmed by the GLC–MS–SIM technique using a resolution of 5000 (10% valley definition). The relative ratios of the relevant fragment ions (as mentioned under GLC–MS Confirmation) in the standards and the unknowns agreed well. For example, during the GLC–MS–SIM confirmation of HMNTHZ isolated from a fried Polish coil sausage the relative ratios of the three fragment ions (at m/z 117.0123, 132.0483, and 162.0463) for the unknown and the standard (both analyzed as *O*-methyl ether) were 35:100:52 and 38:100:47, respectively.

In summary, we have developed a reliable and sensitive method for the determination of two difficult-to-analyze nonvolatile *N*-nitroso compounds in various smoked foods. It is hoped that other researchers will find the method useful for this purpose.

ACKNOWLEDGMENT

We thank P.-Y. Lau of this laboratory for performing the mass spectrometric confirmation of HMNTHZ in one sample.

LITERATURE CITED

Fine, D. H.; Huffman, F.; Rounbehler, D. P.; Belcher, N. M. Analysis of *N*-Nitroso Compounds by Combined High-Performance Liquid Chromatography and Thermal Energy Analysis. *IARC Sci. Publ.* **1976**, *No. 14*, 43–50.

Havery, D. C. Determination of *N*-Nitroso Compounds by High-Performance Liquid Chromatography with Post-Column Reaction and a Thermal Energy Analyzer. *J. Anal. Toxicol.* **1990**, *14*, 181–185.

Ho, J. L.; Wisneski, H. H.; Yates, R. L. Determination of *N*-Nitrosodiethanolamine in Cosmetics by High Pressure Liquid Chromatography Thermal Energy Analysis. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 800–804.

Hotchkiss, J. H. A Review of Current Literature on *N*-Nitroso Compounds in Foods. *Adv. Food Res.* **1987**, *31*, 53–115.

Kubacki, S. J.; Havery, D. C.; Fazio, T. Nonvolatile *N*-Nitrosamine Investigations: Methods for the Determination of *N*-Nitrosamino Acids and Preliminary Results of the Development of a Method for the Determination of *N*-Nitrosodipeptides *N*-Terminal in Proline. *IARC Sci. Publ.* **1984**, *No. 57*, 145–158.

Mandagere, A. K. Smoke-Related *N*-Nitroso Compounds in Cured Meat Systems. Ph.D. Thesis, Michigan State University, East Lansing, MI, 1986; Chapter II, pp 73–98.

Massey, R. Analysis of *N*-Nitroso Compounds in Foods and Human Body Fluids. In *Nitrosamines*; Hill, M. J., Ed.; Horwood: Chichester, U.K., 1988; Chapter 2, pp 2.1–2.47.

Massey, R. C.; Crews, C.; Dennis, M. J.; McEweeny, D. J.; Startin, J. R.; Knowles, M. E. Identification of a Major New Involatile *N*-Nitroso Compound in Smoked Bacon. *Anal. Chim. Acta* **1985**, *174*, 327–330.

Pensabene, J. W.; Fiddler, W. Effect of *N*-Nitrosothiazolidine-4-carboxylic Acid on Formation of *N*-Nitrosothiazolidine in Uncooked Bacon. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 1077–1080.

Preussmann, R.; Stewart, B. W. *N*-Nitroso Carcinogen. In *Chemical Carcinogens*; Searle, C. E., Ed.; ACS Symposium Series 182; American Chemical Society: Washington, DC, 1984; pp 643–828.

Sen, N. P. Analysis and Occurrence of *N*-Nitroso Compounds in Foods. In *N-Nitroso Compounds: Biology and Chemistry*; Bhide, S. V., Rao, K. V. K., Eds.; Omega Scientific Publishers: New Delhi, 1990; pp 3–18.

Sen, N. P.; Kubacki, S. J. Review of Methodologies for the Determination of Nonvolatile *N*-Nitroso Compounds in Foods. *Food Addit. Contam.* **1987**, *4*, 357–383.

Sen, N. P.; Donaldson, B. A.; Seaman, S.; Iyengar, J. R.; Miles, W. F. Recent Studies in Canada on the Analysis and Occurrence of Volatile and Non-Volatile *N*-Nitroso Compounds in Foods. *IARC Sci. Publ.* **1978**, *No. 19*, 373–393.

Sen, N. P.; Seaman, S. W.; Baddoo, P. A.; Weber, D. Determination and Occurrence of 2-(Hydroxymethyl)-*N*-nitrosothiazolidine in Fried Bacon and Other Cured Meat Products. *J. Agric. Food Chem.* **1989**, *37*, 717–721.

Sen, N. P.; Seaman, S. W.; Weber, D. Mass Spectrometric Confirmation of the Presence of *N*-Nitrosopyrrolidine in Instant Coffee. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 325–327.

Sen, N. P.; Baddoo, P. A.; Seaman, S. W.; Weber, D. 2-(Hydroxymethyl)-*N*-nitrosothiazolidine-4-carboxylic Acid in Smoked Meats and Bacon and Conversion to 2-(Hydroxymethyl)-*N*-nitrosothiazolidine During High-Heat Cooking. *J. Food Sci.* **1991a**, *56*, 913–915.

Sen, N. P.; Seaman, S. W.; Baddoo, P. A.; Weber, D.; Malis, G. Analytical Methods for the Determination and Mass Spectrometric Confirmation of 1-Methyl-2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid and 2-Nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid in Foods. *Food Addit. Contam.* **1991b**, *8*, 275–290.

Tricker, A. R.; Kubacki, S. J. Review of the Occurrence and Formation of Nonvolatile *N*-Nitroso Compounds in Foods. *Food Addit. Contam.* **1991**, in press.

Tricker, A. R.; Perkins, M. J.; Massey, R. C.; Bishop, C.; Key, P. E.; McWeeney, D. J. Incidence of Some Nonvolatile *N*-Nitroso Compounds in Cured Meats. *Food Addit. Contam.* **1984**, *1*, 245–252.

Umano, K.; Shibamoto, R.; Fernando, S. Y.; Wei, C.-I. Mutagenicity of 2-Hydroxyalkyl-*N*-nitrosothiazolidines. *Food Chem. Toxicol.* **1984**, *22*, 253–259.

Received for review July 19, 1991. Revised manuscript received November 5, 1991. Accepted December 6, 1991.

Registry No. HMNTCA, 99452-46-7; HMNTZ, 92134-93-5.