# COMMUNICATIONS

## Analysis of Nonionic Nonvolatile *N*-Nitroso Compounds in Foodstuffs

Simple analytical procedures are described which are capable of quantitatively detecting microgram per kilogram levels of nonionic nonvolatile *N*-nitroso compounds in complex foodstuffs such as cooked bacon, dried beef, luncheon meat, oily and nonoily fish, and liquors. High-pressure liquid chromatography is employed, in combination with the *N*-nitroso specific Thermal Energy Analyzer.

The carcinogenicity of *N*-nitroso compounds is well established (Magee, 1971). The majority are expected to be derivatives of amines which because of either thermal instability, polarity, or high molecular weight are not amenable to gas chromatographic (GC) procedures. Many papers have been published over the last few years on establishing the distribution of *N*-nitroso compounds in the environment. However, because of a lack of adequate analytical instrumentation, the data have been restricted to the 14 or so GC amenable *N*-nitroso compounds; nothing is currently known about the distribution of the majority of this important class of compounds. We have recently developed (Fine et al., 1974) a new method of detection for *N*-nitroso compounds based upon the catalytic breaking of the N–NO bond and the subsequent detection of the nitrosyl radical. The new technique, called thermal energy analysis (TEA), is uniquely selective to the nitroso functional group and is sensitive to picogram quantities (Fine et al., 1975b). The TEA has been interfaced to both a gas chromatograph, TEA–GC (Fine and Rounbehler, 1975), and a high-performance liquid chromatograph, TEA–HPLC (Fine et al., 1976).

Techniques for the analysis of *N*-nitroso compounds at the <10 μg/kg level in foodstuffs can be divided into three broad, nonexclusive categories: volatiles, ionic nonvolatiles, and nonionic nonvolatiles. Each category requires different extraction, concentration, and chromatographic methodologies. TEA techniques are available for the volatiles using TEA–GC (Fine et al., 1975a). Procedures for the ionic nonvolatiles have not yet been developed. We report here on the first procedures capable of quantitating nonionic, nonvolatile *N*-nitroso compounds at the 10 μg/kg level in complex foodstuffs such as liquors, fish, cooked bacon, and meat products.

### EXPERIMENTAL SECTION

**Materials Used.** Solvents were redistilled in glass. *N*-Nitrosobenzylphenylamine, *N*-nitrosocarbazole, and *N*-nitrosocarbaryl were supplied by Oak Ridge National Laboratory, *N*-nitrosoatrazine was supplied by EPA, Athens, Ga., and *N*-nitrosodiphenylamine was supplied by Fisher Scientific. All *N*-nitroso compounds were recrystallized before use, and all gave a molar response on the TEA. Samples were spiked with a single compound in 25 μl of dichloromethane or ethanol. Identical recoveries were obtained using both solvents. Commercially available food samples were used throughout.

**High-Pressure Liquid Chromatograph.** The instrument was assembled using a Waters Associates Model 6000 high-pressure pump and a Waters Associates Model U6K injector. Detection was achieved with an *N*-nitroso compound specific Thermal Energy Analyzer (Thermo Electron, Model 502), equipped with a TEA–HPLC interface (Fine et al., 1976). The TEA cold trap was used at −98 °C. Parallel uv and TEA chromatograms were obtained by connecting a uv detector (Waters Associates, Model 440) in series with the TEA, and allowing the column eluent to flow sequentially through first the uv and then the TEA detectors.

**(a) Liquor Samples.** Samples (100 ml) were extracted three times with 50 ml of dichloromethane (DCM). The DCM extracts were combined and dried over 150 g of sodium sulfate. The DCM was removed from the sodium sulfate by filtration and the sodium sulfate washed twice with 50 ml each of DCM. The combined filtrates were reduced to dryness under vacuum. The residue was made up to 0.5 ml with DCM.

**(b) Nonoily Fish Tissue.** Sliced tissue (25 g) was frozen in liquid nitrogen and transferred to a stainless steel Waring Blendor containing liquid nitrogen. The fish was blended in the liquid nitrogen slurry until a milklike paste was obtained. The slurry was transferred to a 250-ml beaker, using liquid nitrogen to wash out the blender. The liquid nitrogen was allowed to evaporate, leaving a fine powder. Ten grams of powdered anhydrous sodium sulfate and 150 ml of DCM were added, and the mixture stirred for 5 min. The mixture was allowed to stand for 10 min and stirred again. The mixture was then filtered through fluted paper containing 15 g of anhydrous powdered sodium sulfate. The residue was washed sequentially with two 25-ml portions of DCM and the combined filtrates concentrated under vacuum at ambient temperature.

The concentrate was transferred quantitatively to a 2-dram vial with DCM. The DCM was removed under a stream of nitrogen. The residue was dissolved in 0.5 ml of the HPLC carrier solvent. For oily fish an additional step was necessary. After removal of DCM from the extract in the 2-dram vial, the oily residue was washed sequentially with three 2-ml aliquots of acetonitrile. The combined acetonitrile washes were placed in a 2-dram vial and taken to dryness under a stream of nitrogen. The temperature in the final concentration step was not allowed to exceed 35 °C.

**(c) Cooked Bacon, Luncheon Meat, Dried Beef.** An aliquot of chopped tissue (25 g) was placed in a stainless steel Waring Blendor containing liquid nitrogen, and blended to a uniform slurry. Acetonitrile (100 ml) was added in small portions (approximately 10 ml) with additional liquid nitrogen. Powdered anhydrous sodium sulfate (5 g) was added and the residual mixture warmed to ambient temperature. The acetonitrile slurry was gravity filtered through granular anhydrous sodium sulfate. The filter cake was washed sequentially with two 40-ml portions of acetonitrile. The combined acetonitrile extracts were washed sequentially with 15- and 5-ml portions of 2,2,4-trimethylpentane. If phase separation did not occur

Table I. Determination of Nonvolatile *N*-Nitroso Compounds at the 10 μg/kg level

| Compd determined | Matrix | Anal. method | % recovery |
|---|---|---|---|
| *N*-Nitroso-diphenylamine | Vodka | a | 60 |
| *N*-Nitroso-atrazine | Vodka | a | 60 |
| *N*-Nitrosobenzyl-phenylamine | Fresh white fish | b | 60 |
| *N*-Nitrosobenzyl-phenylamine | Fresh cod fish | b | 90 |
| *N*-Nitroso-carbaryl | Cooked bacon | c | 60 |
| *N*-Nitroso-carbazole | Cooked bacon | c | 50 |
| *N*-Nitrosobenzyl-phenylamine | Cooked bacon | c | 80 |
| *N*-Nitrosobenzyl-phenylamine | Dried beef | c | 60 |
| *N*-Nitrosobenzyl-phenylamine | Deviled ham | c | 70 |

rapidly it was facilitated by cooling to 0 °C. The acetonitrile extract was taken to dryness under vacuum at 35 °C, and the residue transferred to a 3-dram vial with DCM. The DCM was removed under a stream of nitrogen and the residue dissolved in 0.5 ml of HPLC carrier solvent.

**Chromatographic Conditions.** A 4 × 300 mm Waters Associates μ-Porasil column was employed. Between 1 and 5% v/v acetone in 2,2,4-trimethylpentane was used as the carrier solvent at a flow of 2 ml/min. Because acetone absorbs at the 254-nm wavelength of the uv detector, 1% 2-propanol in dichloromethane was used as the solvent system for obtaining parallel uv–TEA chromatograms.

## RESULTS AND DISCUSSION

Recoveries routinely obtainable by these three procedures are presented in Table I. Triplicate recoveries of *N*-nitrosobenzylphenylamine from cooked bacon gave an average recovery of 80 ± 9%. The signal-to-noise ratio of the TEA–HPLC chromatogram for each quantitative recovery was greater than 10:1. Response on the blanks was less than twice the noise and was not subtracted. However, due to the specificity of the TEA–HPLC system nonresponding material may be present in the extracts at concentrations sufficiently high to alter the retention time on the chromatographic column without affecting the response of compounds of interest. Addition of standards to blank extracts confirmed that retention times were altered in the presence of an extreme excess of nonresponding material. Confirmation of retention times in actual samples was achieved by spiking the sample extracts.

The specificities of the methods described here to *N*-nitroso compounds are due primarily to the TEA–HPLC system. When the effluent from the HPLC is monitored by a conventional high-performance uv absorbance detector the benefit of the TEA specificity is clearly illustrated. The chromatograms of 20 μl of a 1 μg/ml standard ethanol solution containing 20 ng of *N*-nitrosobenzylphenylamine are shown in Figure 1c on the uv detector and in Figure 1d on the TEA detector. Figures 1a and 1b are the chromatograms of 20 μl of a cooked bacon extract, with both detectors on the same sensitivity scales as for Figures 1c and 1d (0.01 AUF for the uv, and ×4 for the TEA). Because there are so many uv absorbing species in cooked bacon, the bacon extract causes the uv detector to be off scale on the 0.01 AUF scale for most of the chromatogram. By comparison, the TEA

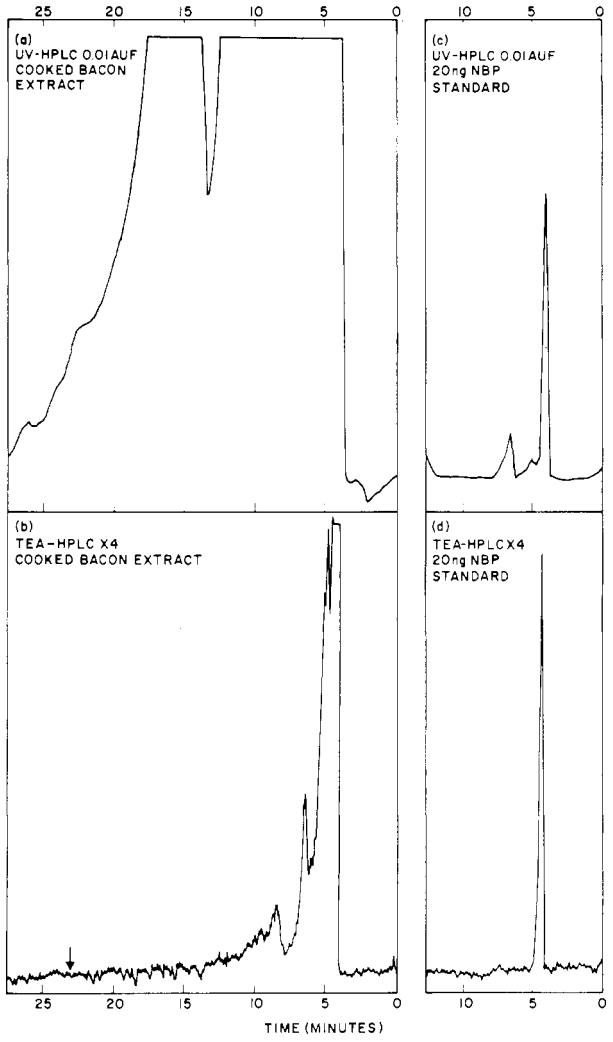


Figure 1. Parallel uv-TEA HPLC chromatograms using 1 ml/min of 1% 2-propanol in dichloromethane on a μm Porasil column: (a and c) uv traces on the 0.01 AUF sensitivity scale; (b and d) TEA traces on the ×4 attenuation. Figures 1a and 1b are the chromatograms for 50 μl of a cooked bacon extract. Figures 1c and 1d are the chromatograms for 20 μl of standard solution containing 1 μg/ml of *N*-nitrosobenzylphenylamine.

detector shows only an initial peak, followed by two smaller peaks. Although both detectors have similar sensitivity to a uv absorbing compound like *N*-nitrosobenzylphenylamine, only the TEA detector is capable of making use of the high sensitivity when chromatographing crude environmental samples. The initial peak shown on the TEA detector in Figure 1b can, under different solvent conditions, be resolved into several smaller peaks, including *N*-nitrosopyrrolidine and *N*-nitrosodimethylamine, plus several other peaks. The unknown TEA peaks detected in cooked bacon extracts have not yet been isolated or identified.

Figure 2a is the TEA–HPLC chromatogram of 50 μl of a cooked bacon extract, using 1% acetone in 2,2,4-trimethylpentane as the carrier solvent. Figure 2b is the chromatogram of the same bacon sample which had been spiked with 10 μg/kg of *N*-nitrosobenzylphenylamine. Recovery was 84%. The large initial TEA peak shown in Figure 1b is not observed under these chromatographic conditions.

## CONCLUSION

The analytical procedures described here make possible, for the first time, the determination of nonionic nonvolatile

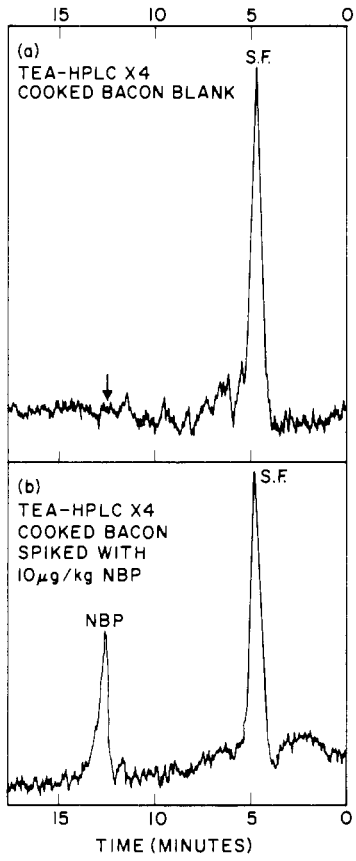


Figure 2. TEA-HPLC chromatograms of cooked bacon extracts using 2 ml/min of 1% acetone in 2,4,6-trimethylpentane on a μm Porasil column, with the TEA on attenuation ×4: (a) the cooked bacon blank; (b) a similar following recovery of 10 μg/kg of *N*-nitrosobenzylphenylamine.

*N*-nitroso compounds in complex foodstuffs and other environmental samples. Application of these procedures will provide previously unobtainable data on environmental levels of nonionic nonvolatile *N*-nitroso compounds. Several as yet unknown TEA responsive compounds are found to be present in all liquor, fish, and meat samples analyzed so far. The identity of the unknown compounds needs to be determined, because, given the specificity of the TEA, there is a high probability that some of the unknowns may correspond to *N*-nitroso compounds. Although the ability to evaluate environmental levels of *N*-nitroso compounds is now greatly extended, determination of total *N*-nitroso levels must still await adequate methodologies for highly ionic *N*-nitroso compounds.

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# Mechanism of Degradation of Thiofanox in Aqueous Solutions

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, is stable in acidic and neutral solutions. At pH 10, it undergoes a two-step oxidation to its sulfone derivative with subsequent hydrolysis of the sulfone. The rate of hydrolysis of the sulfone derivative at pH 10 was determined at 4, 25, and 45 °C, but no direct hydrolysis of P at pH 10 was detected.

Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime, is an effective systemic and contact insecticide. Metabolic studies in plants (Whitten and Bull, 1974; Chin et al., 1976), animals (Tallant and Sullivan, 1974), and soils (Duane, 1974) indicated that the two-step oxidation of P to its sulfoxide derivative ($P_1$), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, and sulfone derivative ($P_2$), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone *O*-[(methylamino)carbonyl]oxime, is the primary mode of degradation. In most cases, only $P_2$ was found to undergo hydrolysis to its oxime ($O_2$), 3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime. Although hydrolysis of $P_1$ to its oxime ($O_1$), 3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime, was occasionally detected, hydrolysis of P to its oxime (O), 3,3-dimethyl-1-(methylthio)-2-butanone oxime, was seldom detected in meaningful quantities in the environmental studies of P (Holm et al., 1975). The purpose of this investigation was to study the mechanism of the degradation of P in aqueous solutions by comparing the