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## Note

# Photolysis of volatile nitrosamines* at the picogram level as an aid to confirmation

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Many nitrosamines (NAs) are potent animal carcinogens[1]. They also have been suggested as a potential source of environmental carcinogens for humans. Considerable worldwide research effort is underway to isolate, quantitate, and identify these substances in foodstuffs. The usual method of separating volatile NAs from other food components involves extraction, distillation, solvent partitioning, and column and gas–liquid chromatography (GLC)[2]. Nitrogen-specific detectors, such as the Coulson or Hall and the alkali flame ionization detectors, have been used for NA determination, but they lack specificity. Fine *et al.*[3] developed a thermal energy analyzer (TEA) which can be used as a detector for GLC[4] and high-pressure liquid chromatography[5]. The TEA is claimed to be specific for NAs since the N–NO group is cleaved by pyrolysis, then the liberated NO radical is reacted with ozone to yield a chemiluminescent response[6]. Despite this, a number of non-nitrosamines, particularly nitro compounds, give a weak TEA response[7]. In addition, because of the great sensitivity of the instrument, picogram quantities of NAs can be detected at concentrations too low for mass spectral (MS) confirmation when present in samples derived from natural products. As an aid in determining whether these small GLC–TEA peaks are NAs, we developed a procedure based on the rapid and complete photolytic decomposition of NAs by ultraviolet (UV) light[8,9]. The method and results obtained in the analytical nitrosamine system used in our laboratory are reported herein.

## EXPERIMENTAL

### *Reagents*

All solvents and chemicals were reagent grade or better and were used without further purification. They were checked by GLC to ensure the absence of impurities.

### *Photolysis*

Standard thin-walled melting point capillary tubes (1.6–1.8 × 100 mm) (Kimax-51*** or equivalent) were thoroughly washed with acetone, dried, and sealed at

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* *Note*: Nitrosamines are potentially carcinogenic and should be handled with care.
** Agricultural Research Service, U.S. Department of Agriculture.
*** Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

one end prior to use. Twenty microliters of solutions, containing either the following seven NAs: dimethyl-, methylethyl-, diethyl-, methylbutylnitrosamine, nitroso-piperidine, -pyrrolidine, and -morpholine, or sample extract, were introduced into the tubes using a 25-μl syringe. This volume was selected for convenience although 10–60 μl could be used. After sample introduction, the tubes were immersed in liquid nitrogen and sealed with a small flame. Incomplete closure could result in solvent evaporation during photolysis and give poor results. The tubes were then placed in a Chromato-Vue cabinet on a platform *ca.* 5 cm from a 366-nm UV lamp. Sample controls consisted of tubes covered with aluminium foil or other opaque material placed adjacent to those being photolyzed. After the appropriate time interval, the tubes were opened and the solutions were removed with a 10-μl syringe.

*Apparatus*

Approximately 6 μl of the test material were injected into a Varian-Aerograph Model 2740 gas chromatograph interfaced with a TEA. The 275 cm × 3 mm stainless-steel column was packed with 16% Carbowax 20M-TPA on 60–80 mesh Gas-Chrom P. The column was programmed from 130 to 190° at 4°/min with an argon carrier flow of 85 ml/min. The injector port temperature was 190°. The TEA operating conditions were: catalytic pyrolyzer at 450°, cold trap at −150° and a vacuum of 2 mm Hg.

*Nitrosamine analysis*

The procedure for the determination of volatile NAs is a modification of the multidetection method described by Fazio *et al.*[10], with only minor changes in the quantity of some of the chemicals used. Fried bacon or other product (25 g) was used with 0.5 μg of methylethylnitrosamine (MENA) added as an internal standard prior to digestion with methanolic KOH or direct extraction with methylene chloride. A 1.0 ml methylene chloride concentrate was obtained for GLC separation and quantitation. For these experiments 40 μl of concentrate were used for the photolyzed and unphotolyzed (control) tubes and the rest was reduced to *ca.* 0.1 ml for confirmation by GLC–high-resolution MS under conditions previously reported[11].

## RESULTS AND DISCUSSION

An average of 50% of the seven NAs disappeared within 10–15 min when we subjected a methylene chloride solution containing 0.5 ng/μl of each NA to the experimental conditions for photolysis. After 60 min, none of the NAs could be detected.

To determine the effect of solvent, we photolyzed 1 ng/μl of the same NAs in hexane, methanol, or water. The NAs in hexane disappeared completely within 60 min. After 90 min, NA decomposition ranged from 98 to 100% in methanol and from 82 to 92% in water. For photolysis in the latter two solvents under these experimental conditions, a minimum of 2-h exposure to UV light is recommended. The photolytic stability of NAs, particularly in aqueous solution, has been reported[12,13]. While the addition of acid may facilitate NA photolytic degradation[14,15], we found it was not necessary for our purposes.

We photolyzed decreasing concentrations of the seven NAs in methylene

chloride to determine a minimum level at which this technique could still be utilized. Decomposition was complete at all concentrations. We measured a minimum of 12 pg/$\mu$l of each NA, or a total of 250 pg, under our usual GLC and detection conditions. An increase in injection size and lower TEA attenuation would significantly increase the sensitivity of this method.

We then applied this photolysis procedure to samples derived from fried bacon. The chromatograms of the same sample unphotolyzed and following UV irradiation (Fig. 1) show three peaks of interest. The peak at 4.5 min is the MENA internal standard and is equivalent to 60% recovery of this NA. Methylethylnitrosamine is not normally found in fried bacon. The other peaks correspond to the retention times of dimethylnitrosamine (DMNA) and nitrosopyrrolidine (NPy). The concentration of the two NAs is equivalent to 2 $\mu$g/kg DMNA and 6 $\mu$g/kg NPy with respect to the quantity of fried bacon used. After photolysis, the three peaks disappeared thus providing additional presumptive evidence of the presence of these NAs. Under our conditions, 3 $\mu$g/kg or greater of NA can be confirmed by MS with a high degree of reliability, except when the sample contains a large amount of interfering material that prevents the determination of the exact mass of the parent ion. In this particular sample, NPy could easily be confirmed by MS but the small amount of DMNA would make MS confirmation extremely difficult without additional sample scale-up and clean-up. This method has been successfully applied to the analysis of volatile NAs in other food products and types of materials including fried bacon drippings, cured sausage, fish and cheese products, tobacco smoke condensate, deionized water, gastric contents, and saliva.

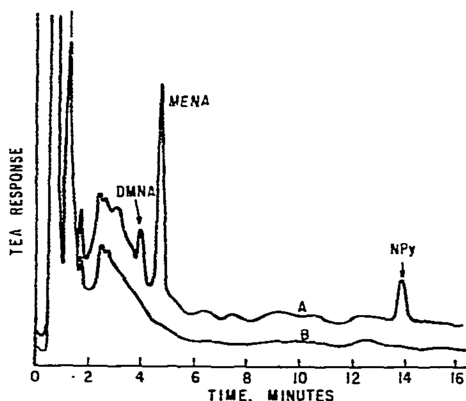


Fig. 1. GLC–TEA chromatograms of 5.8 $\mu$l methylene chloride concentrate from fried bacon containing 50 pg/$\mu$l DMNA, 300 pg/$\mu$l MENA and 144 pg/$\mu$l NPy. A, unphotolyzed; B, after photolysis (vertical scale offset for A and B).

The sample solutions must be transparant to UV light. The efficiency of denitrosation is adversely affected by the presence of highly colored material and suspended matter. Fortunately, the use of an added NA to obtain recovery data for the separation and isolation procedure is a good indicator to determine the completeness of the denitrosative degradation. We occasionally observed skewed shaped peaks, having a retention time greater than NPy in photolyzed but not in unphotolyzed fish samples.

These peaks, probably secondary reaction products, do not significantly affect the utility of the photolysis procedure. We also found several samples that gave a TEA response in the GLC region where volatile NAs would be expected to elute. These peaks did not disappear upon photolysis, thus indicating that they were probably not due to NAs. The identification of these compounds is currently under investigation.

For very low levels of NAs that cannot be confirmed by MS or for researchers without mass spectrometer facilities, photolysis at 366 nm of a small portion of the sample concentrate offers a simple, rapid, and sensitive method for the presumptive evidence of NAs. It is desirable to obtain MS confirmation of NAs where possible, because this is still the best method for determining the identity of these compounds at present. However, photolysis affords an alternate means of validating the positive response of the TEA, providing information concerning the possible presence of heterofore unknown NAs, and identifying the response due to non-NAs.

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