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# A Rapid and Simple Method for the Determination of Volatile N-Nitrosamines in Biological Materials

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Trace amounts of volatile N-nitrosamines (NNA), potent carcinogens, have been detected in normal human faeces (WANG et al. 1978), urine (KAKIZOE et al. 1979) and blood (FINE et al. 1978, LAKRITZ et al. 1980)

In vivo formation of NNA was also discussed associated with the intake of nitrite and nitrate. However, the determination methods for NNA in these biological samples have customarily employed many steps of clean up procedure, because formerly used detectors such as electron capture or flame thermionic detector have poor selectivities toward NNA. Although a thermal energy analyzer combined to a gas chromatograph (TEA-GC) has considerable selectivity, it is necessary to examine its applicability to biological materials, particularly when some of clean-up procedures are abbreviated.

We reported here a TEA-GC method for NNA in biological samples, liver, kidney and blood, and this method is a modification method for foods (MAKI et al. 1978).

## MATERIALS AND METHODS

Chemicals: Nine dialkylnitrosamines were commercialy available (Kanto chemical); N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitroso-di-iso- propylamine (NDi-PA), N-nitrosomethylbutylamine (NMBA), N-nitrosodi-n-propylamine (NDPA), N-nitrosodi-iso-butylamine (NDi-BA), N-nitrosodi-n-butylamine (NDBA), N-nitrosopiperidine (NPIP), N-nitrosomorpholine (NMOR), Other chemicals were used as received.

Apparatus: Thermal Energy Analyzer (TEA 502 type) was combined to a gas chromatograph to determine 9 NNA. A gas chromatographic column was packed with 20%-Versamide 900 on Chromosorb W acid washed 60-80 mesh(3m x 3mm i.d. glass tube). The column was used isothermal at 180°C, injection port 220°C, with Argon carrier gas flow rate at 60mL/min. The furnace of TEA was used at 450°C. The TEA-GC was operated under conditions similar to those published previously (MAKI et al. 1978).

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Proposed Procedure

Sample (5~10 g, or 0.1~1.0 g for dried powder) was placed in a 50mL stopped centrifuge glass tube, and 10mL of 15%-ammonium sulfamate in 10%-sulfuric acid solution is added to it. The mixture is extracted with dichloromethane (15, 10 10mL) by the use of shaking machine (Kayagaki shaker, MS-1 type) and centrifuge (3000rpm, 3 min ). The separated dichloromethane (lower layer) is collected, dried over anhyrous $Na_2SO_4$, and concentrated to 1 mL in a Kuderna Danish evaporator at 35°C in vaccuo.

An aliquot of the sample and standard solution (~3 ng each of NNA) is injected onto the column. A standard curve is prepared by plotting response (peak height) versus concentration.

Chemicals and all sample blanks used in this study were checked for NNA and detected to be free from in any of the samples in the present method.

## RESULTS AND DISCUSSION

Figure 1 illustrates chromatograms of rat control liver with and without the addition of 9 NNA.

The peaks in A represented to 0.5 ng of each NNA, and B represented no interferences were observed in these samples, though, no particular clean up step such as distillation or column chromatography was involved.

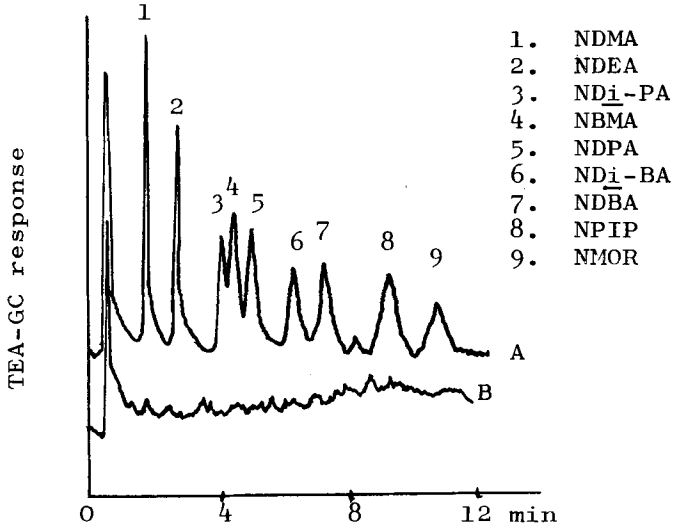


Figure 1. Chromatograms of TEA-GC

A: Control liver with addition of nine NNA
B: Control liver

Table 1 shows the average recovery efficiencies were 85.1% for liver, 92.1% for kidney, and 80.2% for blood for each 0.5 μg level of 9 NNA.

TABLE 1

Recovery efficiency of nine N-nitrosamines from biological materials

| Compound | Liver | Kidney | Blood |
|---|---|---|---|
| NDMA | 80±2.3% | 85±2.9% | 85±3.1% |
| NDEA | 81±2.8 | 91±3.5 | 80±4.0 |
| NDi-PA | 85±4.7 | 96±4.8 | 82±4.5 |
| NBMA | 80±1.7 | 93±2.4 | 76±1.3 |
| NDPA | 91±0.6 | 96±0.4 | 83±4.0 |
| NDi-BA | 97±3.0 | 101±2.1 | 71±4.6 |
| NDBA | 92±2.9 | 102±4.5 | 72±3.8 |
| NPIP | 88±0.8 | 93±6.0 | 91±4.1 |
| NMOR | 72±0.4 | 72±0.4 | 82±3.4 |
| Total | 85.1 | 92.1 | 80.2 |

Results were expressed as a mean±S.E. from five observations

Good recoveries of NNA in 3 different organs of rat were obtained in the present method. A low temperature (35°C) in the concentration procedure gave higher recoveries than those previous method (FINE et al. 1975). Concentration at 65°C according to their description resulted in lower recovery of NDMA;40% for NDMA.

When some of the biological materials were extracted with dichloromethane for NNA , acidic media are superior to neutral or alkaline media because of less emulsification. Furthermore, it is well known that the promotion of NNA formation was involved in the presence of halogen ions (FAN et al 1973, BUGLASS et al. 1974), therefore, we adopted sulfuric acid in place of hydrochloric acid (FINE et al. 1977).

This method has sensitivities of 0.01 ng for NDMA, NDEA and 0.03 ng for other NNA, and minimum detectable levels in biological samples were 0.2 ng per kg for NDMA, NDEA and for other NNA were 0.6 ng per kg.

Samples of liver, kidney, and blood of Wister female rats which were fed ad libitum the basal diet

for 4 months, were analyzed in order to examine the applicability of the procedure, and to ascertain the levels of NNA in them. The NNA levels were below the detection limits and no interferences were observed in analyses.

A rapid and simple extraction NNA from biological materials and resulted high sensitivity of thermal energy analyzer being combined to a gas chromatograph were described here in this paper. It is recommended that this method can be used to monitor exposure to NNA; for examination whether the biological samples were contaminated at high or low levels of NNA. This method enables 20 analyses of the different biological samples of rat within a day.


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