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Note

Use of isomers in the detection and estimation of volatile nitrosamines by combined high-resolution mass spectrometry–gas chromatography

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Several methods have been used for the analysis of trace amounts of volatile N-nitroso compounds. These consist principally of (i) use of a conventional gas chromatograph (GC) employing a nitrogen-sensitive detector[1–3], (ii) use of a chemiluminescence analyser[4,5], and (iii) a GC coupled with a high-resolution mass spectrometer[6–8]. The last method has been used most widely and usually involves peak matching techniques to monitor the different nitrosamines.

In the method described here, focussing of the mass spectrometer is carried out by first inserting a sample of an isomer of the nitrosamine to be examined and locating the mass ion isomeric to that of the nitrosamine. Samples containing the suspected nitrosamine are injected into the GC and the mass spectrometer acts as a specific ion detector at a given retention time. The use of isomers in this way has advantages of safety over the use of the highly carcinogenic nitrosamines themselves for focussing purposes.

EXPERIMENTAL AND RESULTS

All of the isomers and nitrosamines except MEN* were obtained commercially and used without further purification. Standard solutions of each nitrosamine in spectroscopic grade hexane were stored in a refrigerator and solutions (10 ppm) were made up from these before injection of standard amounts into the GC.

Gas chromatography–mass spectrometry

Throughout the determinations a Perkin-Elmer Model F11 gas chromatograph with a hydrogen flame ionisation detector was used, the carrier gas being helium at a flow-rate of 12 ml/min. The column employed for all the nitrosamine determinations consisted of 10% DEGA on 80–100 mesh Chromosorb W (9 ft. × 1/8 in. O.D.). The temperature of the column was varied for each nitrosamine determination and was in the range 100–180°. In all cases 1–2 μl injections of nitrosamine solutions in hexane were made into the column.

The effluent from the column passed through a split (15:1), with the major portion flowing into the GC–mass spectrometer interface (Watson-Biemann separator)

* For abbreviations of N-nitrosamines, see Table I.

by way of a heated (200°) metal transfer line. A valve was positioned between separator and ion source so that the passage of effluent from the column into the ion source could be controlled. In all cases the bulk of the solvent from any injection was excluded from the mass spectrometer.

The mass spectrometer used was a Perkin-Elmer Hitachi RMU 7L double focussing instrument set at a resolution of 10,000 (30% valley). The ion source temperature was maintained at 200°, the ionisation energy being 75 eV and the accelerator voltage being 4800 V.

Use of isomers for nitrosamine determinations

In this particular study seven volatile N-nitroso compounds have been examined and they are listed below. In each case, in order to focus on the exact mass ion of a nitrosamine, an isomer was chosen, having the same molecular weight or exhibiting an ion isomeric to the same mass as in the nitrosamine spectrum. All the isomers had to be relatively volatile (*i.e.*, generally melting below 100°), stable at 200–250° and giving a good recorder response for the particular ion under examination. The abundances of the ions examined for each nitrosamine are given in Tables I and II.

TABLE I

VOLATILE N-NITROSAMINES

N-nitroso derivative of	*Ion monitored (m/e)*	*Abundance of ion (% base peak)*
Dimethylamine (DMN)	74	87.8
Diethylamine (DEN)	102	57.9
Di-*n*-propylamine (DPN)	130	21.9
Di-*n*-butylamine (DBN)	84*	80.0
Methylethylamine (MEN)	88	68.4
Pyrrolidine (NOpyr)	100	50.4
Piperidine (NOpip)	114	48.4

* Fragment ion.

TABLE II

ISOMERS OF VOLATILE NITROSAMINES

*Nitrosamine**	*Positive ion monitored*	*Isomer*	*Abundance of isomer ion (% base peak)*
DMN	$C_2H_6N_2O$	Methyl urea	64.0
DEN	$C_4H_{10}N_2O$	Butyric acid hydrazide	15.0
DPN	$C_6H_{14}N_2O$	N-(2-Aminoethyl)-morpholine	1.6
DBN	$C_8H_{19}N$**	Tri-*n*-butylamine	6.5
MEN	$C_3H_8N_2O$	Ethyl urea	32.4
NOpyr	$C_4H_8N_2O$	Cyclopropane carboxylic acid hydrazide	17.7
NOpip	$C_5H_{10}N_2O$	1-Piperazine carboxaldehyde	32.7

* Abbreviations as in Table I.

** Fragment ion in spectrum of DBN and of tri-*n*-butylamine.

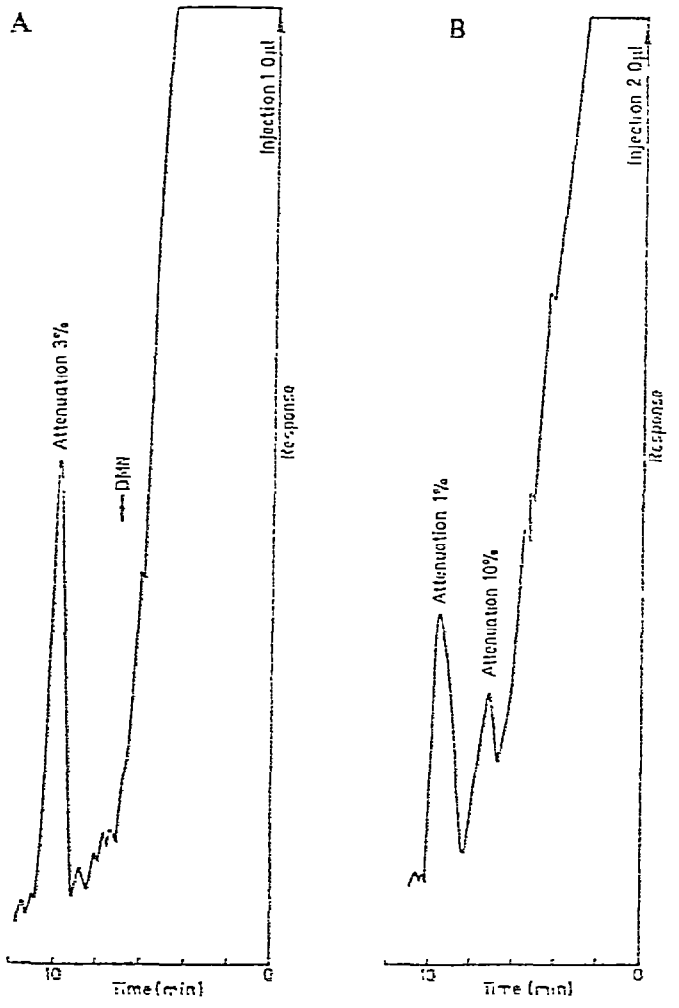

Fig. 1. Mass spectrometric response at stated attenuations of (A) without and (B) with the addition of 2.0 ng DMN at $m/e = 74.0480$ in the gas chromatography of a concentrated food extract in hexane. Injection volumes: (A) 1.0 µl; (B) 2.0 µl.

A few milligrams of the isomer were introduced into the mass spectrometer by way of a heated glass inlet system and the magnet was precisely focussed on the required ion. A 1-µl aliquot of an authentic nitrosamine was then injected into the GC and the detector responses were recorded on a chart recorder. The solvent was excluded from the mass spectrometer and the valve controlling access to the mass spectrometer was only opened after the peak due to solvent had diminished. It was possible to observe directly any response for nitrosamine coming from the column after the valve had been opened. The response for 10 ng of the nitrosamine was recorded and the retention time noted: in between each run the focussing of the magnet was carefully checked to ensure that the mass spectrometer had remained focussed accurately at the correct *m/e* value. In this way, it was possible to compare

TABLE III

RETENTION TIMES OF N-NITROSAMINES ON MASS SPECTROMETER

*Nitrosamine**	*Column temperature (°C)*	*Retention time (min)*
DMN	100	7.50
MEN	110	7.25
DEN	120	6.50
DPN	140	6.00
DBN	170	6.57
NOpyr	180	6.00
NOpip	180	5.25

* Abbreviations as in Table I.

the response at the given retention time of the suspected nitrosamine with that obtained from 10 ng of the authentic nitrosamine. Using this method the limit of detectability for a given nitrosamine was about 1 ng/μl*. The measurement of response was facilitated by the use of an attenuator unit (constructed by our In-Line Process Control Section) which could precisely cut down the incoming signal by a given fraction. In some cases interfering peaks occurred near the specific retention time for the nitrosamine being monitored, especially in the case of DMN. Fig. 1 shows the MS response at an *m/e* value of 74.0480 in two cases, A being an injection of 1 μl of a sample containing suspected nitrosamine and B a repeat injection of the same sample which had been spiked with DMN itself. The trace shows resolution of the two peaks, indicating that the major peak in B with the correct *m/e* value was not due to DMN. The attenuation figures refer to percentages of the incoming signal being detected on the recorder output of the mass spectrometer.

The retention times for the nitrosamines are noted below. The column temperature for DMN was adjusted to give a relatively long retention time so as to improve any interference effects.

DISCUSSION

The use of isomers for specific ion focussing is suitable for analysis of volatile N-nitrosamines and has the advantage over oscilloscope peak matching methods in that a permanent record is made of the results without having to use photographic methods. Monitoring of a nitrosamine ion by this method is also more specific than examination of the NO^+ ion[8], which can also be produced in the fragmentation of other compounds such as those containing a nitro group. Whilst a resolution of 70,000 is required to distinguish between *m/e* values for the molecular ion of DMN and that of $^{29}Si(CH_3)_3$ (ref. 9), a value of 10,000 is usually considered to be adequate.

Warning note

N-Nitrosamines are very carcinogenic compounds and a strictly laid-down procedure should be adopted for all aspects of experimental work involving their use.

* In the case of DPN, the response for 10 ng was smaller, so the limit of detection was about 3 ng/μl.

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