CHROM. 5017

The gas chromatographic determination of nitrosamines at the picogram level by conversion to their corresponding nitramines

We have had occasion to examine the response to a series of nitrosamine standards in hexane of flame ionisation and electron capture detectors fitted to a gas chromatograph. After initial analysis the solutions were inadvertently allowed to stand in direct sunlight for several hours. When these standards were reinjected we found that the peak associated with nitrosamine response had decreased slightly in size and a second peak of longer retention time had appeared in every case. The response of this new species to electron capture was some 200 times greater than its response to flame ionisation.

An examination of each new species using gas chromatography–mass spectrometry showed in every case that its molecular weight was 16 mass units higher than that of the original nitrosamine. High resolution mass spectrometry confirmed that this was due to an extra oxygen atom in the molecule. It therefore seemed possible that the new species could be the corresponding nitramine.

We have now prepared a series of nitramines from their corresponding nitrosamines using a modification of the oxidation technique proposed by EMMONS[1] and have examined their gas chromatographic properties.

Experimental

Reagent. Peroxytrifluoroacetic acid (PTFA). Place 4–5 ml of redistilled methylene chloride in a 10 ml volumetric flask and carefully add 0.4 ml 85–90% w/w hydrogen peroxide by pipette. Slowly add by pipette 2.5 ml redistilled trifluoroacetic anhydride, swirl gently and allow to stand in an ice bath for 5 min. Allow the contents of the flask to attain room temperature, then dilute to volume with redistilled methylene chloride. Prepare fresh reagent every day.

Oxidation technique. Place a 1 ml aliquot of a solution of nitrosamine in methylene chloride in a stoppered test tube. Add 0.1 ml PTFA reagent, mix by swirling and allow to stand for 3½ h. Add 2 drops of distilled water, shake gently and leave for 1 min. Add excess calcium carbonate by spatula (0.25 g). When effervescence ceases add 0.25 g powdered anhydrous sodium sulphate, shake gently and allow to settle. Examine the solution by electron capture gas chromatography.

Results and discussion

Using this technique we have determined the efficiency of conversion, retention times and the relative response to nitrosamine of a flame ionisation detector and to the corresponding nitramine by an electron capture detector.

Efficiency of conversion. This was studied for 50 μg amounts of nitrosamine and the efficiency of conversion was calculated by comparing peak areas for nitrosamine and corresponding nitramine by flame ionisation gas chromatography. A mean of five determinations is reported in each case.

Retention times. These were studied on a 5 ft. PEG 20 M column run isothermally at 140°.

Relative responses. The response in terms of both peak heights and peak areas

TABLE I

THE EFFICIENCY OF CONVERSION OF NITROSAMINE TO NITRAMINE

Nitrosamine	*Conversion (%)*
Dimethyl	86
Methyl ethyl	85.5
Diethyl	84.5
Methyl-isopropyl	76
Di-isopropyl	72
Di-*n*-propyl	84
Di-isobutyl	82.5
Di-*n*-butyl	83
N-Nitrosopiperidine	25
N-Nitrosopyrrolidine	85

TABLE II

RETENTION TIMES OF NITROSAMINES AND CORRESPONDING NITRAMINES

Nitrosamine	*Retention time (min)*	*Retention time of corresponding nitramine (min)*
Dimethyl	4.0	9.8
Methyl ethyl	4.9	11.25
Diethyl	5.5	12.4
Methyl isopropyl	5.8	12.65
Di-isopropyl	7.15	15.05
Di-*n*-propyl	9.5	20.5
Di-isobutyl	10.45	20.65
Di-*n*-butyl	20.2	42.9
N-Nitrosopiperidine	22.5	39.6
N-Nitrosopyrrolidine	25.25	57.65

TABLE III

INCREASE IN SENSITIVITY OBTAINED USING EC DETECTION OF NITRAMINES

Original nitrosamine	*Increase in sensitivity for nitramine*	
	By peak areas	*By peak heights*
Dimethyl	1150	545
Methyl ethyl	620	372
Diethyl	438	215
Methyl isopropyl	243	141
Di-isopropyl	119	65
Di-*n*-propyl	245	125
Di-isobutyl	215	110
Di-*n*-butyl	205	91
N-Nitrosopiperidine	183	102
N-Nitrosopyrrolidine	255	98

of nitrosamines by flame ionisation detection and of the corresponding nitramines by electron capture detection were determined. Increases in sensitivity of between 100 fold and 1100 fold, depending on the particular nitrosamine studied, were obtained for nitramines using electron capture as shown in Table III.

The smaller increase in sensitivity obtained from peak heights is a reflection of the increased retention time of the nitramines.

It would therefore seem that the technique of oxidation of nitrosamines to their corresponding nitramines, coupled with the use of electron capture GLC, offers a means of improving the sensitivity of detection of the former by several orders of magnitude and of thus achieving the detection levels required for biological purposes.

Addendum

After preparing this communication we learned from Dr. N. P. Sen of the Food and Drug Directorate, Ottawa that he has submitted a paper based on the same basic idea to the *Journal of Chromatography* (51 (1970) 301).

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1 W. D. EMMONS, *J. Am. Chem. Soc.*, 76, (1964) 3468.

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Experience with an electrolytic "ninhydrin reactor"

A commercial unit for electrically producing a hydrindantin–ninhydrin reagent for automated amino acid analyzers was very recently marketed (Sondell Scientific Instruments, Inc., Palo Alto, Calif., U.S.A.)*. The device uses a nickel–platinum couple and offers many worthwhile advantages. Claims include improved color yields yet with substantial savings in materials and time by using one-fifth the usual ninhydrin concentration and by eliminating waste through improved stability of ninhydrin stock solution. Absence of tin salts and precipitates reportedly eliminates cuvette clean-ups and costly coil plugging, decreases baseline noise and analyzer down-time, and minimizes calibration runs. The reactor is "...designed to function with any amino acid analyzer" and, assuming appropriate adapters and fitting are at hand, is "...simple to install and operate".

This note is to share experience with other potential users who also may find that eliminating all the interferences which inactivate the unit is easier said than done.

* Mention of trademark or company names is for information purposes and does not constitute preferential endorsement by the U.S. Department of Agriculture.