Semiquantitative Analysis of Low Levels of Volatile Nitrosamines by Gas Chromatography–Mass Spectrometry

A method is described for the analysis of volatile nitrosamines in food using a high-resolution mass spectrometer coupled to a gas chromatograph. An ion, characteristic of each nitrosamine, is chosen and the gas chromatograph effluent is monitored by the spectrometer (at a resolution of 10,000) for any compound whose mass spectrum contains the characteristic ion. The amount of a nitrosamine is estimated from a knowledge of the sensitivity of the mass spectrometer to that nitrosamine. Recoveries of a range of nitrosamines, added to food, have been measured at 10, 2, and 1 ppb.

Recently we described a method (Telling *et al.*, 1971) for identifying volatile nitrosamines using gas chromatography and monitoring the chromatograph effluent for compounds whose high-resolution mass spectrum contained an ion at mass 30 of composition NO^+. It was possible to detect 0.2 μg of an individual nitrosamine in this way. Any compound giving a response was subsequently identified by recording low-resolution mass spectra. However, measurement of recoveries from samples to which nitrosamines had been added showed that as much as 0.5 μg of a nitrosamine could be required to give a sufficiently intense low-resolution mass spectrum for positive identification because of interferences from coextracted materials. As the amount used in the determination was $^1/_{10}$th of the total extract, 0.5 μg of a nitrosamine corresponded to 20 ppb of the original sample (250 g), assuming 100% recovery. It was found that the practical limits ranged from 25 to 65 ppb (0.625 to 1.625 μg injected on the column) depending on the nitrosamine. Although this technique could be used as a screening test, it could not be used for the quantitative measurement of nitrosamine content.

We also pointed out that it was possible to identify certain specific nitrosamines at a level of 10 ppb (0.25 μg injected on the column) by monitoring the gas chromatograph effluent for the molecular ion or any other characteristic ion of the nitrosamine. We now wish to describe the application of the latter technique to the semiquantitative analysis of nitrosamines and report developments which enable us to identify nitrosamines at a level of 1 ppb (0.025 μg injected on the column).

EXPERIMENTAL

Increased sensitivity has been achieved by a modification to the mass spectrometer. The value of the grid resistor of the electrometer stage of the signal amplifier has been increased by a factor of 10, resulting in a tenfold increase in signal strength for the same number of ions at the collector. The signal-to-noise ratio is also improved by this factor since the noise is not appreciably increased at the low bandwidth (20 Hz) used when monitoring a single mass at high resolution. In practice, sample losses on the gas chromatograph and in the gas chromatograph–mass spectrometer interface prevent the full increase being realized at the lower limit. It is now found that the detection limit for the NO^+ ion monitoring technique is 5 to 10 ppb (0.125 to 0.25 μg injected on the column).

Greater sensitivity can be achieved by monitoring for a characteristic ion which is more abundant than the NO^+ ion. The molecular ion is the obvious choice for some of the lower molecular weight nitrosamines. For others with weak molecular ions, *e.g.*, less than 10% relative abundance, an intense fragment ion can be selected if its elemental composition is known. The mass spectrometer is adjusted to detect ions of the same elemental composition as the characteristic ion. This is readily achieved on our mass spectrometer (AEI MS 902) using the peak matching facilities and perfluorotri-*n*-butylamine as the mass calibrant. The resolution of the spectrometer should be at least 10,000 so that most of the possible interfering ions from other compounds are separated from the characteristic ion. A compound eluting from the gas chromatograph is identified as a nitrosamine if it has the correct retention time and possesses the characteristic ion in its mass spectrum. The amount of nitrosamine in the sample can be calculated from a knowledge of the sensitivity of the mass spectrometer for that nitrosamine. Several nitrosamines can be monitored in a single run, provided that the time between the elution of successive nitrosamines from the column is sufficient to allow readjustment of the spectrometer.

The apparatus and extraction procedure have been previously described (Telling *et al.*, 1971).

RESULTS AND DISCUSSION

The compounds chosen for study were *N*-nitrosodimethylamine (DMN), *N*-nitrosodiethylamine (DEN), *N*-nitrosodiisobutylamine (Di-BN), *N*-nitrosodibutylamine (Dn-BN), and *N*-nitrosopyrrolidine (NNPyrr), since they cover a wide range of volatility. The molecular ion of DMN, DEN, and NNPyrr was chosen as the characteristic ion for these compounds; *m/e* 84 was chosen for Di-BN and Dn-BN, as the molecular ion is of low abundance in both spectra. The composition of *m/e* 84 was established as $C_5H_{10}N$ by high-resolution mass spectrometry.

The mass spectrometer sensitivity for each compound was determined by injecting known amounts of the nitrosamine on the column and measuring the height of the characteristic ion peak as it was repetitively scanned and displayed on the mass spectrometer oscilloscope. A standard solution containing all five nitrosamines was used and it was possible to measure the response of each compound during the course of a single determination.

The response of the mass spectrometer should be linearly dependent on the amount of nitrosamine injected on the gas chromatograph, and this was confirmed for all five nitrosamines. Various amounts of nitrosamine in the range 0.010 to 0.5 μg were injected and the mass spectrometer response at each characteristic mass was measured, in turn, as the compounds eluted from the column. The sensitivity of the mass spectrometer for each nitrosamine was calculated from these data. The minimum amount of nitrosamine which can be accurately measured is of the order of 0.025 μg injected

Table I. Percentage Recovery of Nitrosamines Added to a 250-g Sample of Luncheon Meat at the 10, 2, and 1 ppb Levels

Level, ppb, of nitrosamine	10 % Recovery			2 % Recovery			1 % Recovery		
	Max	Min	Mean[a]	Max	Min	Mean[a]	Max	Min	Mean[a]
DMN	94	55	75	88	35	56	Interference could not be measured		
DEN	93	65	82	132	61	102	125	41	96
Di-BN	100	52	78	92	51	72	133	60	98
Dn-BN	73	47	58	80	51	69	106	53	77
NNPyrr	52	29	41	56	27	42	101	30	69

[a] The mean value of at least six determinations.

on the column. This corresponds to 1 ppb of the original sample after allowing for the quantities used in the method. However, these values vary on a day-to-day basis and frequent calibration of the mass spectrometer is necessary. Recalibration is essential if the mass spectrometer conditions are changed, *e.g.*, to record low-resolution spectra between nitrosamine determinations. A single measurement of the five nitrosamines used in this study takes 1 hr. Thus it is possible, in a day, to carry out several such measurements to calibrate the instrument and still analyze two or three samples. The time required to analyze a sample for each of the five nitrosamines is governed by the chromatographic conditions, which were chosen so that the nitrosamines are adequately separated from each other. The analysis of an extract for one or two nitrosamines only can be accomplished in a much shorter time by altering the chromatographic conditions.

The overall procedure was checked by adding all five nitrosamines to a typical food substrate, luncheon meat, and measuring the amount recovered. Percentage recovery values of nitrosamines added to samples at 10, 2, and 1 ppb are given in Table I. There is a wide spread in the data, especially at the lowest level. The low precision of the quantification by gas chromatography–mass spectrometry and variations in recovery in the extraction procedure are mainly responsible for the spread in the data. The recovery values which are greater than 100% arise through measurement errors and not from nitrosamines originally present in the sample, since no nitrosamines were detected in the original samples. The lack of precision of the measurements means that only semiquantitative analyses can be carried out in the 1–5 ppb range. However, the technique still offers a means of qualitative analysis at these levels.

SAFETY NOTE

Nitrosamines are highly carcinogenic compounds and all experimental work should be done in a well ventilated area. Safety gloves should be worn whenever nitrosamines are being handled.

LITERATURE CITED

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Received for review November 26, 1971. Accepted February 7, 1972.

Factor Responsible for Varietal Differences in Aflatoxin Production in Maize

Varietal differences in aflatoxin production in food grains is of considerable practical significance. The present study on seven hybrid varieties of maize indicated marked differences in aflatoxin production. Opaque-2 produced markedly low toxin, while Deccan hybrid produced maximal amounts. The possible reasons for the differences in toxin production have been investigated. Different extracts of the two varieties of maize (Opaque-2 and Deccan hybrid) have been incorporated into a standard synthetic medium and their ability to support toxin production has been examined. A 5% NaCl extract of Opaque-2 could result in almost total inhibition of the toxin production. Further fractionation of the NaCl extract revealed that the inhibitory factor is a protein of low molecular weight. The concentration of this protein is markedly higher in Opaque-2 than Deccan hybrid variety.

Aflatoxin contamination of food grains is now recognized to be a potential health hazard, in view of the known hepatotoxic and carcinogenic effects of the toxin (Goldblatt, 1969). The production of aflatoxin by toxigenic strains of *Aspergillus flavus* L. on different food grains is known to vary widely (Diener and Davis, 1969; Hesseltine *et al.*, 1966; Kriz, 1970). Maximal production of the toxin has been demonstrated on natural substrates such as rice, while meager production is obtained on soya beans (Hesseltine *et al.*, 1966). Varietal differences in aflatoxin production have been observed in peanuts (Rao and Tulpule, 1967; Doupnik, 1969) and in sorghum (Anandam, 1970). Such wide variation in toxin production in different varieties of the same crop could lead to the identification of varieties which