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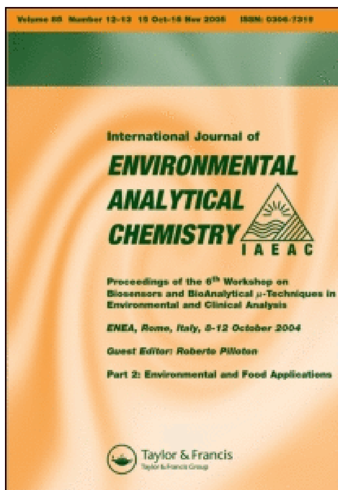

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A Sensitive and Specific Method for the Determination of *N*-Nitrosodimethylamine in Drinking Water and Fruit Drinks

N. P. Sen[a]; P. A. Baddoo[a]; D. Weber[a]; M. Boyle[a]
[a] Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health Canada, Ottawa, Canada

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A SENSITIVE AND SPECIFIC METHOD FOR THE DETERMINATION OF *N*-NITROSODIMETHYLAMINE IN DRINKING WATER AND FRUIT DRINKS

N. P. SEN*, P. A. BADDOO, D. WEBER and M. BOYLE

Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health Canada, Ottawa, Canada, K1A OL2

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A method is described for the determination of *N*-nitrosodimethylamine (NDMA) in drinking water and fruit drinks/juices. It is highly sensitive and specific, and is practically free of artifactual formation. Such formation, in this method, is minimized by distilling or extracting the samples in the presence of sulfamic acid, an excellent inhibitor of *N*-nitrosation. Both gas chromatography—thermal energy analyzer (GC-TEA) and gas chromatography—high resolution mass spectrometry selected ion monitoring (GC-MS-SIM) were used for the determination of NDMA. The minimum detection limits of the two methods were 15 pg/g and 1 pg/g, respectively. The recoveries of NDMA and that of *N*-nitrosodi-*n*-propylamine (NDPA), the internal standard, at 6.6–133 pg/g spiking levels ranged between 74–105%. The efficiency of the method in preventing artifactual formation was verified by carrying out a few analyses with added marker amines such as ethylbutylamine, morpholine, and deuterium labelled dimethylamine. Several samples of contaminated water and fruit drinks contained 0.09–5.5 ng/g levels of NDMA, but those procured from retail outlets or natural sources were negative.

KEY WORDS: *N*-Nitrosodimethylamine, prevention of artifactual formation, drinking water, fruit drinks, gas chromatography thermal energy analyzer determination, gas chromatography-mass spectrometry.

INTRODUCTION

Trace to fairly high levels of *N*-nitrosamines (simply called nitrosamines) have been reported to occur in various foods, mainly cured meat products, certain beverages (e.g., beer and ale), and many industrial and agricultural products (e.g., rubber goods, pesticide formulations)[1–4]. Until recently, however, these compounds have rarely been detected in drinking water. In Canada, the occurrence of *N*-nitrosodimethylamine (NDMA) in the drinking water was first reported in 1989 in the town of Elmira, Ontario[5]. The problem resulted from the contamination of aquifer with industrial pollutants, with subsequent contamination of ground water

*To whom all correspondence should be sent

over a large area. According to one estimate, about 30×10^6 cubic meters of aquifer and 9 $\times 10^6$ cubic meters of ground water have been contaminated with NDMA[5]. Although the details of the findings have not yet been reported in the scientific literature, various media reports suggest that low pg/g to 40 ng/g levels of NDMA were detected in waters taken from several municipal wells in that area. Varying but similar levels of NDMA were also detected in the surface water taken from the adjacent Grand River. Since NDMA is a potent carcinogen in laboratory animals[6], the above findings prompted the Ontario provincial government to close down several municipal wells containing > 14 pg/g NDMA. This interim guideline was adopted by Ontario on the basis of a similar Environmental Protection Agency (EPA) guideline existing in the U.S.A. There was also concern that any foods or beverages prepared with large proportions of such contaminated water might contain traces of NDMA.

These concerns prompted us to develop a sensitive and reliable method for the determination of NDMA in drinking water and fruit drinks that are normally prepared from concentrates with the addition of water. Although suitable analytical methods exist for the determination of NDMA in foods and beverages[7], these methods seemed to be inadequate for the above purpose mainly for two reasons. First, they lacked adequate sensitivity. This was especially true for fruit drinks because most published methods could accommodate only a small sample size (e.g., 10–20 g) which restricted the final detection limit. Secondly, there is always a possibility that traces of NDMA could be produced as an artifact during work-up or analysis of the samples. Such formation is normally prevented or minimized by incorporating a nitrosation inhibitor (e.g., ascorbic acid, sulfamic acid, propyl gallate) in the sample just prior to analysis. Furthermore, it is possible to monitor the extent of this formation by adding a suitable marker amine to the sample and measuring the formation of the corresponding nitrosamine in the final extract. However, the efficiency of these techniques in preventing or detecting artifactual formation at low pg/g levels has not been demonstrated or investigated. Hence the present study was undertaken to develop a method that fulfils all the criteria mentioned above.

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EXPERIMENTAL

Chemical and reagents

Dichloromethane (DCM), distilled in glass, was purchased from Burdick and Jackson, Muskegon, MI. It was redistilled in an all glass apparatus, and tested for NDMA contamination as described previously[8,9].

Marker amine mixture solution, A. Ethyl-*n*-butylamine (EBA) (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) and Morpholine (MOR)(BDH Inc., Ville St-Laurent, Que, Canada). About 125 mg of each amine was dissolved in 25 mL 1 N H_2SO_4, the pH of the solution adjusted to < 2, if necessary, and then extracted with two 25 mL portions of DCM (to remove any nitrosamine impurity). The DCM extract was discarded.

Deuterium labelled dimethylamine hydrochloride, [$(CD_3)_2$ NH.HCl] (D_6-DMA) (Sigma Chemical Co., St Louis, MO) solution 5 mg/mL in 1 N H_2SO_4 containing 1 mg/mL dissolved

ascorbic acid (nitrosation inhibitor). This is referred as marker amine, B_1. A similar solution, termed as B_2, was prepared in which no ascorbic acid was included.

Deuterium labelled *N*-nitrosodimethylamine [$(CD_3)_2$ N-NO] (D_6-NDMA) was purchased from MSD Isotopes, Pointe Claire, Que. Normal NDMA, *N*-nitrosodi-*n*-propylamine (NDPA), and the mixture of 7 volatile nitrosamines[10], all as solutions in ethanol (10 μg/mL) were purchased from Thermedics Detection Inc., Woburn, MA. Appropriate dilute solutions of each of the above were made using either DCM or ethanol (for NDPA internal standard). The following 7 volatile nitrosamines were included in the mixture: NDMA, nitrosodiethylamine (NDEA), NDPA, nitroso-di-*n*-butylamine (NDBA), nitrosopiperidine (NPIP), nitrosopyrrolidine (NPYR), and nitrosomorpholine (NMOR).

All other chemicals were of ACS reagent grade, and the organic solvents were of glass distilled varieties.

Safety note

Since most nitrosamines are carcinogenic to laboratory animals, appropriate precautions should be taken while handling or working with these chemicals.

Cleaning of glassware

All glassware were washed thoroughly using detergents and water, and finally rinsed with acetone and DCM before use. In addition, those used for preparing nitrosamine standards were first soaked overnight in chromic acid solution and then washed as above.

Instrumentation

Gas chromatography—thermal energy analyzer (GC-TEA) system. The details of the instrumentation have been reported previously[11,12]. For the principle and operation of the TEA, which is highly specific for the determination of *N*-nitroso compounds, refer to an article by Fine and Rounbehler[11]. The GC column and the temperature programming were as follows: 10 ft × ¼ in (2 mm I.D.) coiled glass column packed with 10% Carbowax 20 M + 5% KOH on Chromosorb W, High Performance, 100/120 mesh. The column was conditioned overnight at 220°C before use. During GC-TEA analysis the GC oven temperature was held for 2 min at 140°C, and then programmed to 200°C at 6°C/min. The injection port and the GC-TEA interface transfer line were maintained at 200°C and 300°C, respectively. The carrier gas (Ar) flow rate was about 20–25 mL/min. The linearity of the TEA response was checked by carrying out a standard curve for NDMA (30–500 pg injected).

Gas chromatography-mass spectrometric (GC-MS) analysis

Two mass spectrometers, both operating in the electron impact (EI) ionization mode, were used. The first one was a Kratos Concept 1 S high resolution MS. It was interfaced with a

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Hewlett Packard 5890, Series II, GC, and operated in the selected ion monitoring mode (SIM) at a resolution of 10 000 (10% valley definition). Three ions monitored were: m/e 74.0480 ($M^{+\bullet}$ for NDMA), and m/e 80.0851 ($M^{+\bullet}$ for D_6-NDMA), and m/e 130.1106 ($M^{+\bullet}$ for NDPA—the internal standard). A 30 m (i.d., 0.22 mm) DB-Wax (0.25 µm film thickness) fused silica capillary column (J&W Scientific Inc., Folsom, CA, U.S.A.) was used for the GC separation. Other conditions were as follows: The GC oven held for 1.5 min at 60°C, and then raised to 180°C at 10°C/min (held for 6.5 min). The injection port was set at 60°C; the GC-MS transfer line at 250°C, and the carrier gas (He) pressure was maintained at 15 psi.

The other instrument was a VG Analytical MS system (Model 7070 EQ) equipped with a 11/250 VG data system and interfaced to a Varian GC (Model Vista 6000). The front stage of the MS is a conventional double-focusing (forward geometry) instrument used for operation in the EI mode. The instrument was used for both SIM and full scan mass spectrometric confirmation (repetitive exponential scanning; 0.6 sec per decade) of NDMA isolated from various samples. The MS resolutions for the SIM and full scan analysis were 5000 and 1000, respectively.

Method

(a) *Analysis of water*

(i) *Sulfamic acid extraction method.* A 150 mL aliquot of the water sample was taken in a 500 mL separatory funnel, and acidified with the addition of a freshly prepared mixture of 10 mL 1 N H_2SO_4 and 0.1 g sulfamic acid. After 10 min, 1 mL of the marker amine solution 'A' or 'B_1' and 1 mL of NDPA internal standard (20 ng/mL in ethanol) were added, and the mixture was extracted with five 100 mL portions of DCM. The combined DCM extract was washed with 50 mL of 0.3 N or 1 N KOH solution and the aqueous layer was discarded. The DCM extract was then dried by passing through a bed of anhydrous Na_2SO_4 (~ 40 g) placed in a coarse sintered glass Buchner funnel (no suction applied). The filtrate along with a 25 mL DCM rinse were transferred to a Kuderna-Danish (K-D) evaporative concentrator, to which were added 5–10 mg ascorbyl palmitate (AP) (nitrosation inhibitor) and a small piece of boileezer (Fisher Scientific Ltd., Nepean, Ont., Canada). The mixture was concentrated to 0.5 mL as described previously [8]. To avoid losses of NDMA, the extract, at any stage, should not be concentrated using a rotary evaporator or by blowing down in a gentle stream of nitrogen.

(ii) *Alkaline extraction method.* A 180 mL aliquot of the sample was made alkaline by the addition of 5 mL 3 N KOH, and then mixed with either of the marker amine solutions 'A' or 'B_2' and NDPA internal standard as mentioned above. The mixture was extracted with three 100 mL portions of DCM, and the combined DCM extract was washed with 25 mL 0.1 N H_2SO_4 to remove any basic amines that might be present in the sample. The aqueous acidic wash was discarded. The DCM extract was back-washed with 50 mL 0.3 N or 1 N KOH solution, and the organic layer was dried and concentrated as above except that no AP was added during concentration.

(b) *Analysis of fruit drinks/juices*

(i) *Low temperature vacuum distillation (LTVD) from sulfamic acid solution.* A 150 g aliquot of a sample was taken in a 2 L round bottom distillation flask, and mixed

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with a freshly prepared mixture of 20 mL 1 N H_2SO_4, 500 mg sulfamic acid, and 30 mL distilled water. After 10 min, exactly 1 mL NDPA (20 ng/mL in ethanol) and 1 mL of either marker amine 'A' or 'B_1', were added to the mixture, and the solution was distilled under vacuum (flash evaporator) at 45–50°C under subdued light as described previously[13,14]. The aqueous distillate (~ 160 mL) was made alkaline with the addition of 10 mL 3 N KOH, and the mixture extracted with three 100 mL portions of DCM. The combined extract and the condenser rinsing (~ 50 mL DCM rinse) was dried and carefully concentrated, after addition of 5–10 mg AP, to 0.5 mL as above.

(ii) *LTVD from 3 N KOH solution.* A 150 g aliquot of the sample was distilled as above except that the distillation was carried out from a strongly alkaline solution (~ 50 mL water and 33 g solid KOH pellets added and the mixture stirred until the latter dissolved). Appropriate amounts of the internal standard and the marker amines (A or B_2) were added prior to distillation. The aqueous distillate was made alkaline and extracted with three 100 mL portions of DCM. The combined DCM extract was successively washed with 50 mL portions of glycine buffer (pH ~ 2) (to remove amines) and 0.3 N KOH solution (back-wash to remove acids) as described previously[13,14]. The washed extract was dried and concentrated to 0.5 mL as above except that no AP was included.

(c) Determination

(i) *GC-TEA analysis.* A 10 µL aliquot each of the concentrated extracts from steps above were analyzed by GC-TEA using the conditions described earlier. Suitable amounts (5–10 µL) of NDMA (10 pg/µL), NDPA (20 pg/µL), or the other standards (e.g. mixed standards of the 7 volatile nitrosamines) were also analyzed similarly and the amount of NDMA present in the sample extract was calculated by comparison of their relative response. For accurate results, the strength of the NDMA external standard chosen should be such that its peak height (or area) is within ± 50% of that of the NDMA peak in the sample extract[8].

(ii) *GC-MS-SIM analysis.* A 1µL aliquot of the sample extract or of the individual standards (all prepared in DCM) was analyzed using the SIM mode. Before use, separate calibration curves for each standard were carried out to ensure linearity of response for each compound in the desired concentration range.

(d) *Reagent blanks*

With each batch of new reagents or solvents a reagent blank was taken through all the steps of the two methods, and a 10 µL or 1 µL aliquot of the final extract analyzed by GC-TEA or GC-MS-SIM. If a positive result (> 1 ng in 0.5 mL final extract) was obtained, an attempt was made to determine and eliminate the source of the contamination before analyzing any sample.

RESULTS AND DISCUSSION

The methods reported here for the determination of NDMA in water and fruit drinks/juices can be roughly divided into three basic steps: (a) extraction or isolation of NDMA from the

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sample, (b) concentration of the extract, and (c) determination by GC-TEA or GC-MS-SIM. Because of various interfering substances present in the fruit drinks/juices, NDMA could not be directly extracted with DCM and then concentrated to a small volume. It had to be isolated first from the bulk of the matrix by the LTVD method before proceeding to the next steps. The rest of the methodologies are essentially the same for water and the fruit drinks/juices. It should be emphasized, however, that neither of these methods is entirely new. The reliability of the various basic steps (e.g., LTVD, extraction, K-D concentration) and their advantages and disadvantages have already been investigated previously[7,8,14,15]. In this study, we have attempted to combine the various procedures in a most efficient manner so as to obtain maximum possible sensitivity and a minimum of artifactual formation.

Artifactual formation

Previous studies have clearly demonstrated that artifactual formation can indeed occur during analysis of foods[16,17], biological materials[7,18], and various consumer products (e.g., baby bottle rubber nipples)[19]. This happens because nitrosatable amines (e.g., DMA) are ubiquitous in nature and various nitrosating species can originate from chemical reagents, solvents or additives in foods (e.g., nitrite in cured meats). Ambient nitrogen oxides can also form traces of nitrosamines as an artifact in the presence of suitable amine precursors[20]. It has been shown that the addition of various nitrite scavenging agents such as ascorbate, sulfamic acid, propyl gallate, azide or of a strong alkali to the sample at the very start of the analysis greatly minimizes such formation.

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In this study, we have compared the efficiency of sulfamic acid at $pH < 2$, at which it is most effective as a nitrite scavenger, with that of a strong alkali in preventing artifact formation. Three different compounds, EBA, MOR, and D_6-DMA, were used as the marker amines to monitor such formation. The choice of D_6-DMA for this purpose was thought to be more appropriate than the other two because the corresponding nitrosamine, D_6-NDMA, is similar to NDMA in both chemical and chromatographic properties. The nitrosation rates of these two amines (DMA and D_6-DMA) are also very similar. Furthermore, D_6-NDMA does not naturally occur in foods or water, thus reducing the chance of a false-positive result for artifact formation. Since both NDMA and D_6-NDMA eluted at the same time, the GC-TEA technique could not be used for measuring their individual levels. Hence the GC-MS-SIM technique, which is based on detection of the respective molecular m/z ratios, was used for the simultaneous and specific determination of these compounds. The GC-TEA system was, however, useful for detecting artifact formation in cases where MOR and EBA mixture was used as the marker amine. Prior to such experiments, each sample was also analyzed without any added amines to check for the natural levels, if any, of the corresponding nitrosamines.

A few representative data on artifact formation observed during the analysis of water and fruit drinks/juices are presented in Tables 1 and 2. These data clearly demonstrate that for the water samples, the use of the sulfamic acid method followed by an alkaline wash produced much lower levels (mostly < 1 pg/g) of artifact than the alkaline method with or without acid and alkaline washes. Failure to wash the DCM extract with dilute alkali following the initial extraction of the sample from sulfamic acid solution resulted in

formation of traces of nitrosamines (only three shown in Table 1). However, in no case was such an effect observed if an alkaline wash was included. The addition of small amounts of AP during concentration in method 2 (see Tables 1 and 2) prevented further formation, although its effect in this respect was not investigated in detail. With the alkaline method, both the incidence and levels of artifact formation were higher.

A similar conclusion could be drawn from the data regarding the analyses of fruit drinks and juices (Table 2). The sulfamic acid LTVD method produced either none or negligible levels of nitrosamines. The alkaline method, on the other hand, produced 78–341 pg/g levels of artifact formation. Normally such low levels of formation would not be of concern if the

Table 1 Extent of artifactual formation during analysis of water under various conditions

Sample	*Method used*[a]	*Marker amines added*	*Levels (pg/g) of the corresponding nitrosamines formed as an artifact*[b]
Bottled water	1	EBA, MOR	NEBA (113), NMOR (135)
Above water	2	EBA, MOR	(N)[c], (N)
Contaminated water[b] from a food plant	2	EBA, MOR	(N), (N)
Distilled water	1	D_6-DMA	D_6-NDMA (130)
Distilled water	1	D_6-DMA	D_6-NDMA (620)
Distilled water	2A	D_6-DMA	D_6-NDMA (3.7)
Lake Ontario water from point (A)	1A	D_6-DMA	D_6-NDMA (60)
Above water	2A	D_6-DMA	D_6-NDMA (16)
Above water	2	D_6-DMA	D_6-NDMA (<1)
Lake Ontario water from point (B)	1A	D_6-DMA	D_6-NDMA (160)
Above water	2	D_6-DMA	D_6-NDMA (1)
Above water	2A	D_6-DMA	D_6-NDMA (22)
Lake Ontario water from point (C)	1	D_6-DMA	D_6-NDMA (96)
Above water	2	D_6-DMA	D_6-NDMA (6)
Ottawa River water	1	D_6-DMA	D_6-NDMA (<1)
Above water	2	D_6-DMA	D_6-NDMA (<1)
Calabogie Lake water	1	D_6-DMA	D_6-NDMA (10)
Above water	2	D_6-DMA	D_6-NDMA (<1)

[a] Methods used: 1—DCM extraction at an alkaline pH followed by both acid and alkaline washes of DCM extract.

1A—As above but omitting both the washes.

2—DCM extraction from sulfamic acid (pH <2), alkaline wash of DCM extract, concentration with 5–10 mg added AP.

2A—As above extract but the alkaline wash of DCM extract omitted.

[b] All samples were negative for NDMA except this contaminated water sample from a food processing plant contained 155 pg/g NDMA.

[c] N = negative (detection limits, 15 pg/g by GC-TEA and 1 pg/g by GC-MS-SIM).

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Table 2 Artifactual formation of nitrosamines during analysis of fruit drinks/juices using two different methods.

Sample	*Method used*[a]	*Marker amines added*	*Levels (pg/g) of the corresponding nitrosamines formed as an artifact*[b]
Tropical fruit drink	1	EBA, MOR	NEBA (341), NMOR (82)
Above sample	2	EBA, MOR	NEBA (N[c]), NMOR (78)
Apple drink	1	EBA, MOR	NEBA (176), NMOR (257)
Above sample	2	EBA, MOR	N, N
Orange drink	2	EBA, MOR	N, N
Orange juice	2	EBA, MOR	N, N
Apple juice	2	EBA, MOR	N, N
Orange-apricot drink	2	EBA, MOR	N, N
Diet cola	2	EBA, MOR	N, N
Orange-apricot drink (contaminated[d])	2	EBA, MOR	N, N
Apple drink (contaminated[e])	2	EBA, MOR	N, N
Orange drink	2	D_6-DMA	D_6-NDMA (N)
Grapefruit juice	2A	D_6-DMA	D_6-NDMA (31)
Strawberry drink	2	D_6-DMA	D_6-NDMA (N)
Orange drink	2	D_6-DMA	D_6-NDMA (N)

[a] Methods used: 1—Low temperature vacuum distillation from 3 N KOH solution, followed by DCM extraction of basified distillate and both acid and alkaline washes of DCM extract, and concentration without the addition of AP.

2—Low temperature vacuum distillation from sulfamic acid solution (pH < 2), DCM extraction of basified distillate, concentration with the addition of 5–10 mg AP.

2A—Same as 2 except no AP was added during concentration.

[b] All samples were negative for NDMA except the 2 samples marked with superscript d and e which, respectively, contained 5.5 ng/g and 0.49 ng/g levels of NDMA.

[c] N = negative (detection limits, 33 pg/g by GC-TEA and 1 pg/g by GC-MS-SIM).

[d,e] These two were contaminated samples (see above for results).

samples contained relatively high levels (e.g., > 10 ng/g) of preformed nitrosamines[3,20,21] This was, however, considered unacceptable in the present circumstances because of such low levels of NDMA measured in these samples. The two sulfamic acid methods are, therefore, preferable to the alkaline methods for the low level determination of NDMA in water and fruit drinks/juices.

It should be noted that AP and ascorbic acid (in B_1 marker amine) were included only in the sulfamic acid methods. The main purpose was to compare the performances of our improved sulfamic acid methods with those of the older alkaline ones which normally do not employ such inhibitors. A few typical chromatograms from these experiments are shown in Figures 1 to 3. As can be seen from Figure 1 (b versus c), the alkaline method produced artifactual formation of both NEBA and NMOR whereas the sulfamic acid method did not. Also, the former gave a higher value of 0.75 ng/g NDMA in this particular contaminated

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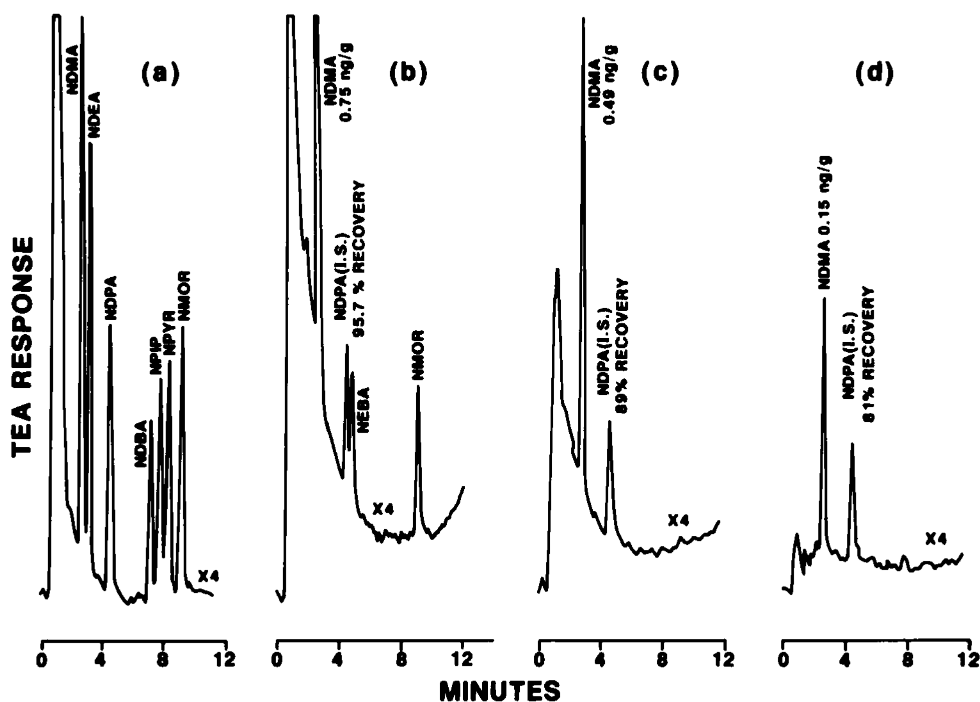

Figure 1 GC-TEA chromatograms: (a) 500–600 pg each of 7 volatile nitrosamines standards (see *Chemicals and reagents* for the list), (b) 10 μL/1 mL final extract of a contaminated apple drink analyzed by the alkaline LTVD method (note artifactual formation of both NEBA and NMOR), (c) 10 μL/1 mL of final extract of above analyzed by the sulfamic acid LTVD method, (d) 10 μL/1 mL final extract of a contaminated water sample analyzed by the sulfamic acid method (note absence of NEBA and NMOR formation; the sample contained 0.15 ng/g NDMA).

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apple drink than the latter which gave a value of 0.49 ng/g. The difference of 0.26 ng/g might be attributed to possible artifactual formation in the alkaline method. Other such examples are presented in Figures 2 and 3.

Accuracy, precision, and sensitivity

The accuracies of the two recommended methods (based on sulfamic acid) were determined by carrying out recovery studies after the addition of 7 volatile nitrosamines to various samples. The recoveries of these nitrosamines (Figure 4) added to water samples at 33 pg/g levels varied between 74–105%. Except for NPYR and NMOR in the sulfamic acid LTVD method, the corresponding data from fruit drinks/juices at 133 pg/g levels ranged between 80–100%. However, the recoveries of NDMA from both water and the fruit drinks in both the methods were consistently high (av. 85%). Furthermore, the recoveries of NDPA, the internal standard, added to each sample at 133 pg/g levels were also excellent. For example,

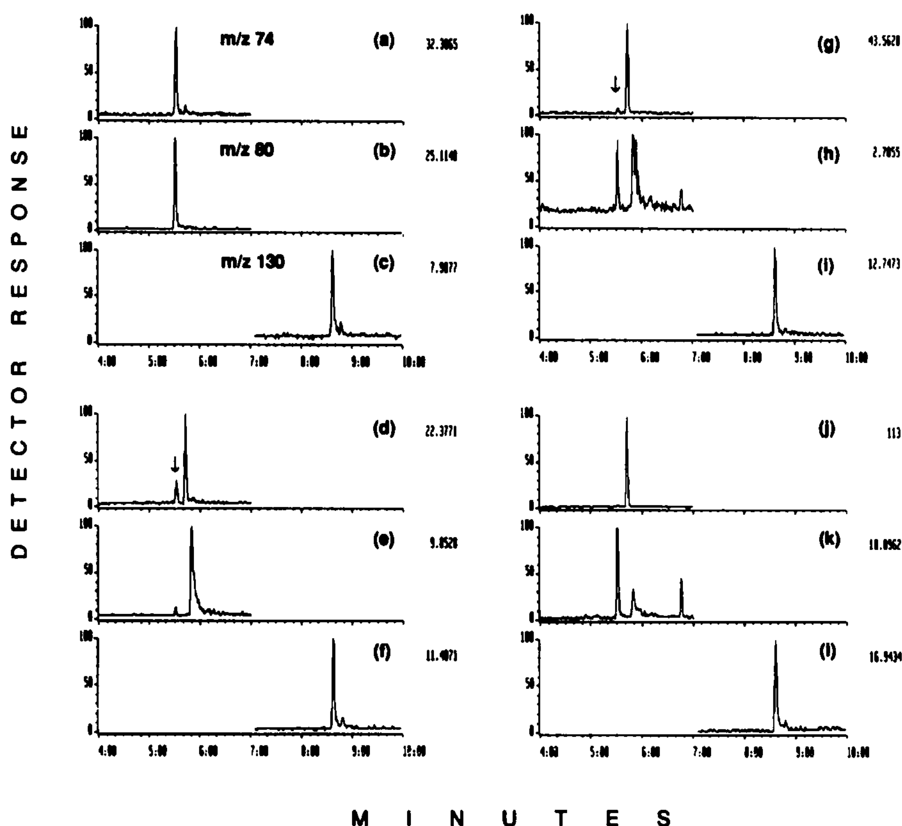

Figure 2 Simultaneous GC-MS-SIM (resolution 5000) for NDMA (m/z, 74), D_6-NDMA (m/z, 80), and NDPA (I.S.) (m/z, 130): (a), (b), (c) for the respective standards, 5 pg of each; (d), (e), (f) are for a control (no D_6-DMA added) water sample (lake Calabogie) analyzed by the sulfamic acid method (note < 5 pg/g levels of preformed NDMA and < 1 pg/g level of D_6-NDMA); (g), (h), (i) are for the same after the addition of D_6-DMA (note 1.2 pg/g levels of preformed NDMA and < 1 pg/g level of D_6-NDMA formation); (j), (k), (l) are for the same water analyzed by the alkaline extraction method after the addition of D_6-DMA (note negligible levels of preformed NDMA but formation of 12.8 pg/g levels of D_6-NDMA). The arrows indicate the retention time of NDMA, and the numbers on the right of each chromatogram represent the peak height counts for the largest peak. Since peak heights of the smaller peaks were automatically normalized on the basis of the largest peak in each, a comparison of apparent peak heights of the NDMA peak between chromatograms can be misleading.

the percentage recoveries of NDPA from 23 samples of water and 33 samples of fruit drinks/juices ranged between 74–130% (av. 91.4 ± 12.4) and 75–101% (av. 92.3 ± 10.3), respectively. These results attest to the excellent performance and good accuracy of the overall method. It should be pointed out that the final results for NDMA, if present, were not corrected for percentage recoveries of NDPA. The latter was used only as a check for overall performance of the method for each analysis. The analysis was repeated if the recovery of NDPA was < 70%.

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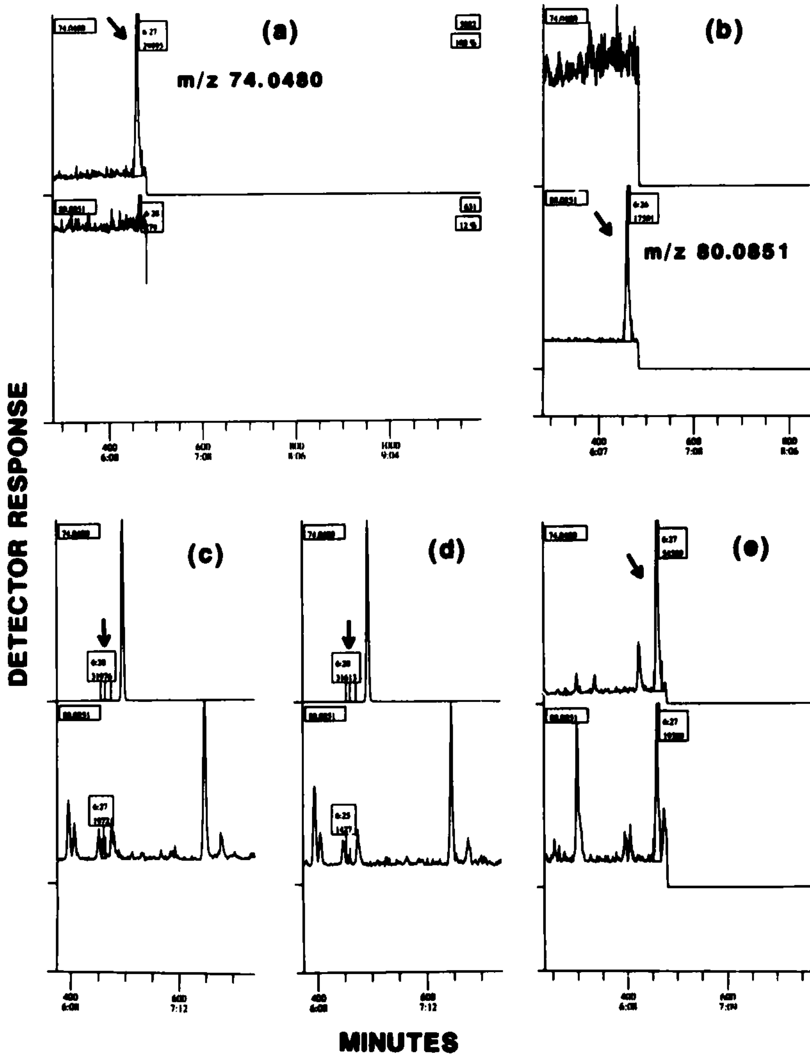

Figure 3 GC-MS-SIM (resolution 10 000) of unspiked and spiked fruit drinks all analyzed by the sulfamic acid LTVD method. (a) 2 pg NDMA, (b) 2 pg D_6-NDMA, (c) an orange drink control (without added D_6-DMA) (note the absence of both NDMA and D_6-NDMA), (d) the same analyzed after the addition of D_6-DMA (note the absence of D_6-NDMA formation), (e) the same spiked with 6.6 pg/g levels each of NDMA and D_6-NDMA (Note: The monitoring was discontinued just after the NDMA peak. This was done to avoid normalization on the basis of the large unknown peak eluting just after NDMA. Also, to avoid overcrowding, the monitoring for NDPA peak has not been shown in these diagrams.)

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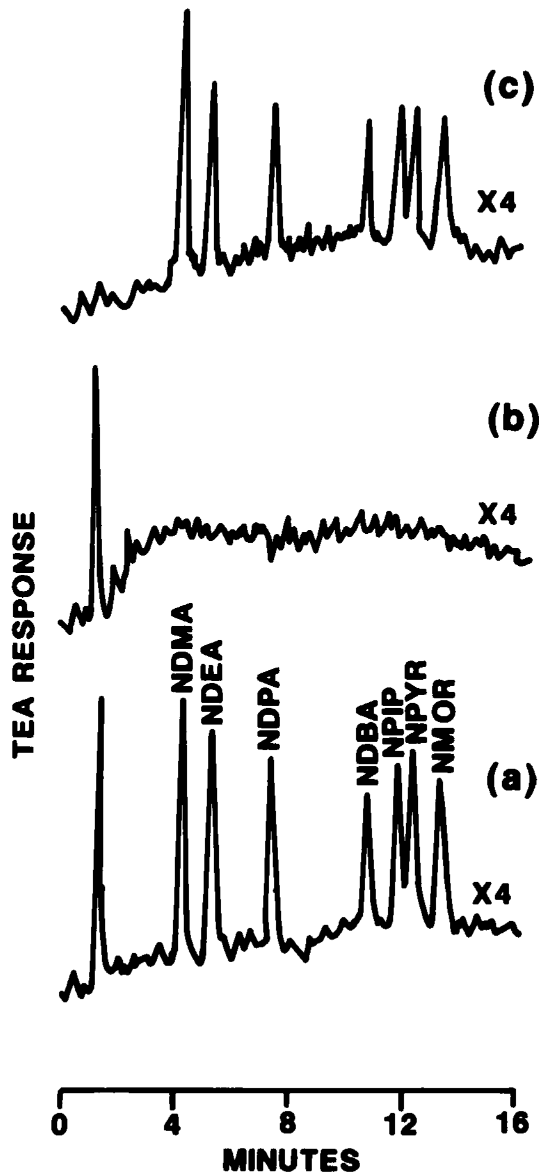

Figure 4 GC-TEA determination of 7 volatile nitrosamines in unspiked and spiked water sample analyzed by the sulfamic acid method. (a) About 100 pg each of the 7 volatile nitrosamines, (b) 10 μL/0.5 mL final extract of an unspiked water sample (note absence of nitrosamines), (c) the same after spiking with 33.3 pg/g levels of each nitrosamines.

As mentioned earlier, these methods were developed on the basis of other related methods that have been thoroughly tested and found to be highly precise[8,22]. For these reasons, the precision of these two methods was not investigated in detail in the present study. However, the limited data suggest that both these methods are reproducible within ± 10%, even at extremely low levels. For example, replicate analyses of 2 water and 3 fruit drink samples for NDMA gave the following values: 0.151 ng/g, 0.155 ng/g and 0.155 ng/g; < 15 pg/g and < 15 pg/g (< detection limit of the GC-TEA method); 0.495 ng/g and 0.584 ng/g; 0.299 ng/g and 0.299 ng/g; and 5.51 ng/g and 6.6 ng/g.

The sensitivity of the two detection methods was determined by carrying out recovery studies at extremely low levels. When a 150 g sample of each of water and a fruit drink was spiked with 1 ng or 5 ng of each of 7 volatile nitrosamines, including NDMA, the recoveries of the nitrosamines ranged between 80–100%. The peak heights, especially those of NDMA, were easily noticeable at the 1 ng/150 g (≈ 6.6 pg/g) level by the GC-MS-SIM technique (Figure 3). From the relative peak height and noise ratios, the minimum detection limits (>3 × noise) of the GC-MS-SIM and GC-TEA techniques were estimated to be 1 pg/g and 15 pg/g, respectively.

NDMA levels in water and fruit drinks/juices

The two sulfamic acid methods were used to determine the levels of NDMA in several samples of the above products (Table 3). Except for the contaminated samples of water and fruit drinks from one food processing plant near Elmira, Ontario, the other samples were negative for NDMA. Likewise, all 8 samples of fruit drinks/juices procured from local retail outlets (in Ottawa) were negative for NDMA. This suggested that NDMA does not occur naturally in such products even at trace levels. The identity of NDMA in 6/9 positive samples of fruit drinks/juices was confirmed by GC-MS-SIM (5000 resolution). In addition, the NDMA isolated from an orange-apricot drink containing 5.51 ng/g level of NDMA was confirmed by full scan mass spectrometry (repetitive exponential scanning at the rate of 0.6 sec per decade). In these spectra, the relative ratios of the major ions (e.g., at m/z 30, 42, 43, and 74) in the isolated NDMA were similar to those in the NDMA standard.

Table 3 Levels of NDMA in some contaminated and normal samples of water and fruit drinks/juices.

Sample	*No. Positive/Total*	*ng/g NDMA in the positive samples*
Bottled water	0/4	
Water from Lake Ontario	0/3	
Water from three other sources	0/4	
Contaminated water from a food processing plant	3/4	0.15, 0.15, 0.16
Various fruit drinks/juices from retail outlets	0/8	
Various contaminated samples of fruit drinks/juices from the above food processing plant	9/9	0.086–5.51[a,b]

[a] The individual levels (ng/g) were as follows: 5.51, 4.03, 4.65, 0.35, 0.84, 0.49, 0.09, 0.39, 0.3.

[b] NDMA in 6/9 samples confirmed by GC-MS-SIM.

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Following the above discovery, the company concerned voluntarily recalled all the contaminated samples of fruit drinks from the market, and destroyed the products. As far as it is known, no further incidences of food or beverage contamination have been reported. We also analyzed 2 samples of cola drinks and 4 of beers from plants located in the same vicinity, but they were either negative or found to contain the expected levels (0.04–0.09 ng/g in the beers) of NDMA which is known to originate from malts used in the preparation of beers and ale[1-3].

In summary, we have developed two reliable methods for the determination of NDMA in water and various fruit drinks/juices that are highly sensitive, accurate, and seem to be free of artifact formation. More importantly, even if any particular sample is found to induce trace levels of formation, the built-in monitoring feature of the method, employing the marker amines, will warn of such a danger to the analyst. The analyst then can take appropriate actions to correct or ignore (if minor) the situation. It is hoped that the methods will be useful to others for research as well as monitoring purposes.

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