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Short Communication

Improving the Chromatographic Analysis of *N*-Nitrosamines in Drinking Water by Completely Drying the Solid Sorbent Using Dry Air

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Abstract

The aim of this study was to develop a method for efficiently extracting eight *N*-nitrosamines (NAs) from chlorinated drinking water using a solid-phase extraction sorbent. This was achieved by completely drying the sorbent using dry air after passing the water through it and before eluting NAs from it. A 500 mL water sample containing NAs was passed through 2.0 g of Carboxen 572. The sorbent was dried by applying a vacuum (-34 kPa) to the sorbent cartridge for 1 h with a silica gel trap connected to the other end of the cartridge. The NAs were then eluted by passing 15 mL of dichloromethane through the cartridge. The dansyl derivatives of the NAs were analyzed by high-performance liquid chromatography with fluorescence detection using a Microsorb-MV Si column and a mixture of water (40%) and acetonitrile (60%) as stationary and mobile phases, respectively. The coefficients of determination (R^2) for five-point linear calibration curves (2-80 ng/L) were 0.9968-0.9997. The relative standard deviations of repeated measurements were mostly less than 5.1%, but were higher for two NAs. The recoveries of all of the NAs when spiked samples were analyzed were > 95.1%, and the estimated method detection limits were 0.5-1.4 ng/L. The method showed much better performance than when the moisture trap was not applied to the cartridge, particularly when the laboratory air had a high level of humidity.

Keywords: chlorinated drinking water, drying sorbent, *N*-nitrosamines, silica gel moisture removal trap, solid-phase extraction

Introduction

There has been much concern about volatile *N*-nitrosamines (NAs) since it was found that *N*-nitrosodimethylamine (NDMA), one of the most commonly detected NAs, can cause liver cancer in rats [1]. The carcinogenicity and frequent occurrence of NDMA in drinking water has led to regulatory limits being proposed for NDMA. The notification level for NDMA in California (USA) is 10 ng/L [2], the maximum acceptable NDMA concentration in Canada is 40 ng/L

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[3], and the World Health Organization guideline value is 100 ng/L [4]. The U.S. Environmental Protection Agency (EPA) has included six NAs – *N*-nitroso-di-*n*-butylamine (NDBA), *N*-nitrosodiethylamine (NDEA), NDMA, *N*-nitrosomethylethylamine (NMEA), *N*-nitroso-di-*n*-propylamine (NDPA), and *N*-nitrosopyrrolidine (NPYR) – in the unregulated contaminant monitoring rule 2 list 2 of contaminants because these NAs are considered to be probable human carcinogens [5]. It has been estimated that a 10^{-5} excess lifetime cancer risk will be caused by NDMA at a concentration of 7 ng/L or by NDEA at a concentration of 2 ng/L.

Finished drinking water has been found to contain NAs at concentrations of up to a few hundred nanograms per liter. NAs in drinking water are mainly produced when water containing ammonia is chloraminated or chlorinated, the secondary amines produced acting as precursors of NAs [6-7]. Gas chromatography or liquid chromatography has usually been used to determine NAs in drinking water [8]. NAs have been extracted by passing drinking water through a solid-phase extraction (SPE) cartridge packed with coconut charcoal or Ambersorb 572, drying the sorbent under vacuum for 10 min [9] or between 30 and 60 min [10-12], then eluting the NAs with dichloromethane (DCM) or a mixture of DCM and methanol. However, no researchers have previously stated that the SPE sorbent was completely dried before the eluting solvent was applied. The atmospheric air drawn through a cartridge during the vacuum drying process will contain moisture, which can lead to the sorbent drying incompletely even when the drying process is long. This will result in the elution process being inefficient and inconsistent.

It is therefore necessary to allow dry air to be drawn into an SPE cartridge during the drying process before the analytes are eluted. It is suggested in U.S. EPA method 521 that a sorbent be dried for 10 min under full vacuum. This has been found to give only moderate recoveries of NDEA, NDPA, and NMEA (84.6%, 81.7%, and 81.8%, respectively), and very poor repeatability for NDEA and *N*-nitrosopiperidine (NPIP) (relative standard deviations of 14% and 20%, respectively) [9]. The method therefore needs to be improved. The aim of the study presented here was to improve the performance of the method for analyzing NAs by passing the air entering the SPE cartridge during the drying process through a silica gel moisture trap.

Materials and Methods

Chemical Reagents and Standard Solutions

Stock solutions of eight NAs (NDBA, NDEA, NDMA, NMEA, NMOR, NPIP, NDPA, and NPYR) in methanol were purchased from Supelco (Bellefonte, PA, USA). The NMEA stock solution had a concentration of 1,000 mg/L, and the other stock solutions each had a concentration of 5,000 mg/L. A 1 mg/L or 5 mg/L standard solution was prepared by diluting the stock solution with methanol. *N*-Nitroso-*n*-butylmethylamine (NBMA) was used as a surrogate standard, and a solution at a concentration of 20 mg/L in methanol was prepared. Blue silica gel was purchased from Showa (Saitama, Japan), acetic acid and sodium bicarbonate were purchased from Daejung Chemicals (Siheung, Korea), hydrobromic acid (47.0-49.0%) was purchased from Wako Pure Chemical Industries (Osaka, Japan), sodium hydroxide was purchased from Kanto Chemical (Tokyo, Japan), and 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride) was purchased from Merck Millipore (Darmstadt, Germany). Carboxen 572, which was used to sorb the NAs, was purchased from Supelco. Acetonitrile, DCM, hexane, and methanol were purchased from Tedia (Fairfield, OH, USA). A certified reference material containing six NAs (NDBA, NDEA, NDMA, NMEA, NDPA, and NPYR) obtained from ERA (Golden, CO, USA) was used to determine the accuracy of the method.

Analytical Procedure

Water samples containing NAs were adjusted to pH 6.5. Each sample was then passed through a prepared SPE cartridge containing Carboxen 572 (2.0 g) following the procedure described in U.S. EPA method 521 [9] with some modifications. The sorbent was conditioned with 20 mL each of hexane, DCM, methanol, and ultrapure water, in that order. A water sample (500 mL) spiked with 1 μL of the surrogate standard solution was then passed through the SPE cartridge at a flow rate of 10 mL/min (Fig. 1a). The sorbent in the cartridge was dried for 60 min by applying a vacuum (-34 kPa) to the lower end of the cartridge while a trap containing blue silica gel (16 g in an impinger) was attached to the upper end of the cartridge (Fig. 1b). The analytes were then eluted by passing 15 mL

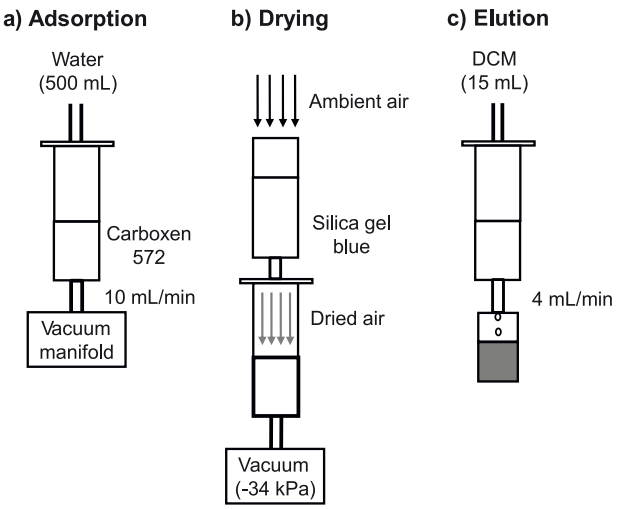

Fig. 1. Analytical procedure used in the study. The adsorbent was dried by attaching a silica gel trap to the top of the solid-phase extraction cartridge and a vacuum to the bottom. It was not necessary to add a Na_2SO_4 trap to the bottom of the cartridge during the elution step to remove water from the eluate.

of DCM through the cartridge at a flow rate of 4 mL/min (Fig. 1c). The analytes in the eluate were then converted into their dansyl derivatives following denitrosation with hydrobromic acid in acetic acid at room temperature for 30 min and reaction with dansyl chloride at 40ºC and pH 10.5 for 30 min [11]. The NAs in a 20 µL aliquot of the prepared sample were then quantitatively determined by high-performance liquid chromatography with fluorescence detection. The mobile phase was a mixture of acetonitrile and water (60:40, v/v), and the flow rate was 1 mL/min. A Waters 474 fluorescence detector (Waters, Milford, MA, USA) used an excitation wavelength of 360 nm and an emission wavelength of 540 nm. The liquid chromatograph used Prostar 210 dual pumps (Varian, Palo Alto, CA, USA), and separation was achieved using a Microsorb-MV Si column (250 mm long, 4.6 mm i.d., 5 µm particle diameter; Agilent Technologies, Santa Clara, CA, USA).

Method Validation

The method validation procedures were conducted when the relative humidity was between 70% and 80%. Calibration curves were constructed using standards at five concentrations between 2 and 80 ng/L. Method precision (repeatability), expressed as the relative standard deviation (in percent), was evaluated by analyzing six replicate samples containing 25 ng/L of each NA. The method detection limits for the NAs were determined by analyzing seven replicate samples containing each NA, following a method based on that described in method 521 [9]. The accuracy with which each compound was able to be determined was evaluated by two methods: by determining the recoveries of NAs that were spiked into samples (each at a concentration of 50 ng/L) and by analyzing the certified reference material in triplicate.

Application to Chlorinated Drinking Water

A total of 60 water samples were collected and analyzed. Twelve samples were collected from each of five faucets in buildings in Chuncheon, Korea, between July and December 2014. Each sample was collected in a 1 L polypropylene container that was wrapped in aluminum foil to stop the sample being exposed to light. Each sample was analyzed within a week of being collected following the procedure described above.

Results and Discussion

Method Validation

Chromatograms of a standard sample (40 ng/L) and a chlorinated drinking water sample are shown in Fig. 2. All of the NA peaks except for the NPYR and NMEA peaks were well separated. Three NAs (NDBA, NDEA, and NDMA) were the dominant contributors to the total NA

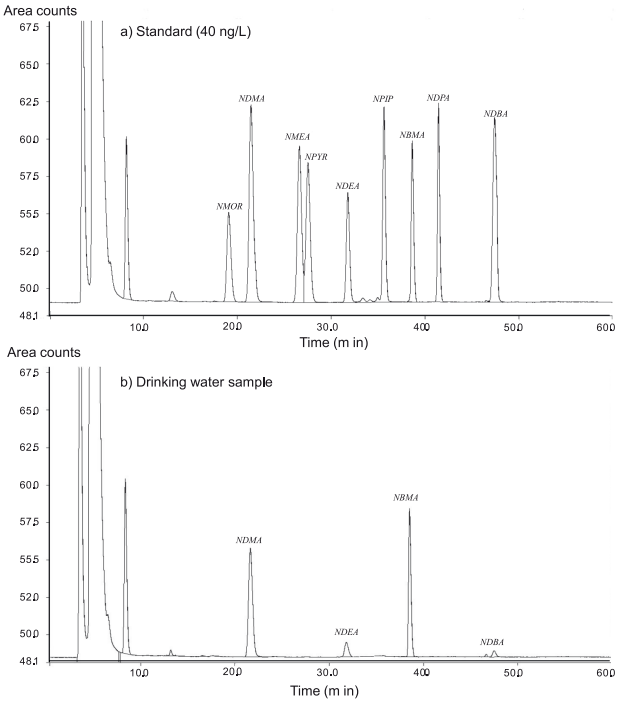

Fig. 2. Chromatograms of a standard (40 ng/L, top) and a chlorinated drinking water sample (bottom). NMOR = *N*-nitrosomorpholine, NDMA = *N*-nitrosodimethylamine, NMEA = *N*-nitrosomethylethylamine, NPYR = *N*-nitrosopyrrolidine, NDEA = *N*-nitrosodiethylamine, NPIP = *N*-nitrosopiperidine, NBMA = *N*-nitroso-*n*-butylmethylamine, NDPA = *N*-nitroso-di-*n*-propylamine, NDBA = *N*-nitroso-di-*n*-butylamine.

concentrations in the drinking water samples. All of the NAs were eluted within 50 min.

The validation results are shown in Table 1. The coefficients of determination (R^2) for the linear calibration curves for all of the NAs were > 0.997. The relative standard deviations for six replicate measurements ranged from 1.6% (for NMOR) to 9.2% (for NDBA). The recovery rates for all of the NAs were > 95%. The method detection limits for most of the NAs were between 0.5 and 1.4 ng/L. These results indicated that the method was satisfactory for quantitatively analyzing NAs in drinking water.

All of the parameters were much poorer All of the parameters were much poorer ($0.9715 \leq R2 \leq 0.9966$; $1.9\% \leq$ relative standard deviation $\leq 19\%$; $54.4\% \leq$ recovery $\leq 88.7\%$; 0.5 ng/L $\leq$ method detection limit $\leq$ 4.4 ng//L) when the moisture trap was not used than when the moisture trap was used. The results for NDEA and NDMA (two of the most common NAs found in chlorinated drinking water) were particularly poor when the moisture trap was not used, the relative standard deviation for NDEA being 19% and the recoveries for NDEA and NDMA being 54.4% and 72.7%, respectively.

The results of the analyses of the certified reference material are shown in Table 1. The NA concentrations determined using the method described here were very close to the certified concentrations in the reference material. The relative standard deviations were reasonable

Table 1. Quality assurance results for the method.

Parameter		NMOR	NDMA	NMEA	NPYR	NDEA	NPIP	NDPA	NDBA
R^2		0.9979	0.9981	0.9985	0.9997	0.9968	0.9991	0.9982	0.9984
RSD (%)[a]		1.6	2.7	3.0	2.5	6.8	2.0	5.1	9.2
Recovery (%)[b]		97.8	100.9	97.8	97.1	100.8	98.1	95.5	95.1
MDL (ng/L)		0.6	0.9	0.6	0.5	1.4	0.5	0.8	0.7
CRM (ng/L)[c]	Reported	-[d]	17.1	11.3	18.8	5.75	-	32.4	7.00
	Measured	-	18.3	10.1	15.8	5.77	-	29.4	7.32

[a]For six replicate analyses with each N-nitrosamine at a concentration of 25 ng/L.
[b]Recovery tests were performed using each *N*-nitrosamine at a concentration of 50 ng/L.
[c]Mean concentration in triplicate analyses.
[d]Not included in the CRM.
NMOR = *N*-nitrosomorpholine, NDMA = *N*-nitrosodimethylamine, NMEA = *N*-nitrosomethylethylamine, NPYR = *N*-nitrosopyrrolidine, NDEA = *N*-nitrosodiethylamine, NPIP = *N*-nitrosopiperidine, NDPA = *N*-nitroso-di-*n*-propylamine, NDBA = *N*-nitroso-di-*n*-butylamine, RSD = relative standard deviation, MDL = method detection limit, CRM = certified reference material.

(2.44% to 8.70%) and the percent errors were satisfactory (1.76% to 14.1%). The results of the analyses of the certified reference material therefore confirmed that the method was reliable.

The results showed that removing moisture from the air entering an SPE cartridge when the cartridge is being dried under vacuum is necessary, particularly if the relative humidity is high. The results suggest that method 521 [9] should be modified to include a moisture-removal trap to allow the analytical performance to be improved.

McDonald et al. [13] dried sorbent cartridges using a gentle stream of nitrogen rather than a vacuum, but they did not mention the flow rate or pressure used, nor whether the sorbent became completely dry. It appears that the sorbent may not have become completely dry, because their recovery rates were lower than the recovery rates achieved using our method and because the recovery rates ranged widely, from 68% at 10 ng/L to 102% at 100 ng/L.

Concentrations of NAs in Chlorinated Drinking Water

The dominant NAs were NDBA, NDEA, and NDMA, and they were detected in 100%, 71.7%, and 100% of the drinking water samples, respectively (Table 2). The mean NDMA concentration was 23.9 ng/L and the range was 4.57 to 62.6 ng/L. The highest NDEA concentration was 23.9 ng/L and the mean was 5.21 ng/L. The highest NDBA concentration was 38.0 ng/L and the mean was 8.24 ng/L. The concentrations of NDEA and NDMA in 68.3% and 95.0% of the samples, respectively, exceeded the concentrations estimated to cause a 10^{-5} excess lifetime cancer risk by the U.S. EPA (2 and 7 ng/L, respectively). This suggested that surveys of NA concentrations in drinking water need to be conducted and NA concentrations need to be controlled to prevent potential health effects occurring in those exposed to drinking water.

Conclusions

Drying the air supplied to the SPE cartridge during the vacuum drying process by passing it through a silica gel trap greatly improved the linearities of the NA calibration curves, the precision of the NA measurements, and the NA recovery rates. Using this sample preparation method when analyzing NAs in disinfected drinking water will therefore allow more reliable analytical data to be produced than is currently the case.

Table 2. Summary of *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), and *N*-nitroso-di-*n*-butylamine (NDBA) concentrations found in chlorinated water.

Compound	Detection rate (%)[a]	Range	Mean	Median	Standard deviation	Samples exceeding 10^{-5} excess cancer risk concentration (%)[b]
NDMA	100	4.57-62.6	23.9	23.0	12.3	95.0
NDEA	71.7	0.70-23.9	5.21	4.51	4.47	68.3
NDBA	100	1.75-38.0	8.24	5.13	6.61	0

[a]Calculated from the number of samples with concentrations higher than the method detection limit.
[b]Percentage of samples exceeding the concentration estimated to cause a 10^{-5} excess lifetime cancer risk by the U.S. Environmental Protection Agency (7, 2, and 60 ng/L for NDMA, NDEA, and NDBA, respectively).

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