Analysis of Nitrosamines in Drinking Water by GC-MS/MS

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Overview

Purpose: A method for the analysis of nitrosamines in drinking water was developed utilizing gas chromatography and quadrupole ion trap tandem mass spectrometry (MS/MS) techniques. Positive chemical ionization was performed, and a study of appropriate reagent gases was performed. Optimal injection techniques and chromatographic conditions were evaluated.

Methods: A 2 µL injection utilizing a pressure and temperature programmable vaporizing injector (PTV) in cold splitless mode offered sample introduction. The GC oven method was optimized to provide sufficient resolution and run length for the compounds. Different reagent gases were evaluated for ionization efficiency, including methane, ammonia, and methanol. An MS/MS method for the ion trap was developed to ensure creation of product ions for each of the target compounds.

Results: The method provided linearity for all components of the method from 1-50 ppb in water. Method development and optimization using a small injection volume allows robust operation of the instrument and maintains source cleanliness.

Introduction

Nitrosamines have been detected in drinking water contaminated with dimethylhydrazine from the production and use of rocket fuel. One member of the nitrosamine family, N-nitrosodimethylamine (NDMA), is a current concern due to its presence in drinking water as a contaminant from chlorination of drinking water (1). Because the nitrosamines are recognized as highly potent potential carcinogens, their presence in drinking water needs to be closely monitored. This led to the development of USEPA Method 521, Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with large volume injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS) (2).

Positive ion chemical ionization (PCI) can be utilized as an ionization technique for mass spectrometry. Different reagent gases have differing proton efficiencies, and this affects the resultant spectra and ions. Reagent gases were evaluated that would more closely match the proton affinities of the ionization agent and nitrosamines. Since there is concern that the dispersion of the ion current into adducts and primary ions may lead to reduced sensitivity in MS^n modes of operation, reagent gas selection focused on a gas that would create primarily the $[M+1]^+$. This ion would then be isolated for the MS/MS experiment. A diagram of the MS/MS experiment is shown in Figure 2.

It should be noted that while methane, which has around a 300 kJ/mol difference in proton affinity when compared to the nitrosamines, was an effective protonating reagent for the nitrosamines, it also led to some adduct formation, principally M+29. The use of dimethyl ether or isobutane as the reagent gas has not been explored at this time. From their relative proton affinities, these gases might also be satisfactory CI reagent gases for this analysis. Ammonia has the closet proton affinity to the amines studied in this work, and so should protonate the compounds of interest with little excess energy. However, the affinities are so close that adducts are expected to form. Figure 3 shows examples spectra for NDEA with the three CI reagent gasses used in this work.

FIGURE 1. The PolarisQ ion trap, shown with TRACE GC Ultra. The instrument was modified to incorporate a liquid CI controller at the entrance to the transfer line. This allowed methanol vapor to enter the source and create the CI plasma.

FIGURE 2. The PolarisQ external ionization source and ion trap. CI occurs in the high-pressure external source, creating ions. The diagram below shows the steps of MS/MS. Ions are injected into the trap. Isolation of precursor ions is followed by application of voltage to induce fragmentation. Product ions are ejected and scanned.

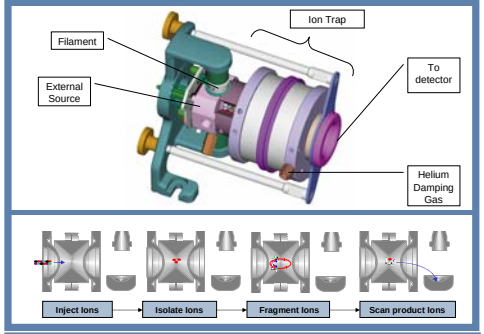


FIGURE 3. Comparison of PCI full scan spectra for NDEA. The top shows the result obtained using methanol as reagent gas. A prominent $[M+1]^+$ mass is shown at *m/z* 103, with little fragmentation and no adduct formation. The middle spectrum reflects use of methane reagent gas. Not only is the $[M+1]^+$ ion present, but adducts at $[M+29]^+$ and $[M+41]^+$ are also present. Finally, the bottom spectrum shows NDMA using ammonia as reagent gas. Like methanol and methane, ammonia gives the $[M+1]^+$ ion. However, the primary ion is an adduct at *m/z* 120, which may disperse ion current. This adduct may also be unstable in the trap.

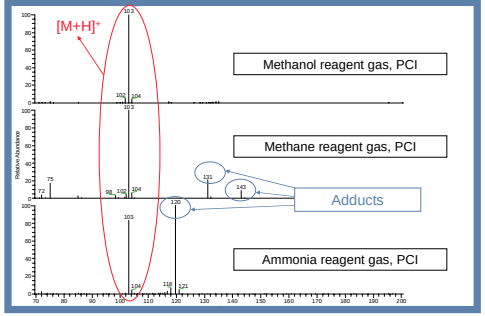


TABLE 1. Nitrosamines and nitroaromatic analyzed on PolarisQ by PCI MS/MS using methane as reagent gas. The precursor ion, retention time and Q voltage are given for each compound.

Retention Time	Compound	Precursor (m/z)	Q value
5.20	NDMA-D6	81	0.225
5.26	NDMA	75	0.225
7.16	NMEA	89	0.225
9.11	NDEA	103	0.225
15.47	NDPA-D14	145	0.225
15.71	NPYR	101	0.225
15.88	NDPA	131	0.225
15.97	NMOR	117	0.225
18.98	NDBA	159	0.225

Methods

Sample Preparation: Nitrosamine standards from Cerilliant Corp (Round Rock, TX) were obtained, from which a primary dilution standard (PDS) at 2 µg/mL was prepared in methylene chloride. NDMA-D6 and NDPA-D14 (Cambridge Isotope Laboratories, Inc., Andover, MA) were used as a surrogate and internal standard respectively. A series of standards ranging from 0.5 to 50 pg/µL was prepared in $MeCl_2$ from the PDS, and the surrogate, NDMA-D6, was added at a final concentration of 20 pg /µL. The internal standard concentration was 10 pg /µL.

Instrumentation: To evaluate the ionization efficiency of three reagent gases, a PolarisQ was adapted to use methanol vapor as the reagent gas. This same PolarisQ was also used to evaluate methane and ammonia as reagent gases. Methane and ammonia reagent gas flow was regulated using the digital reagent gas flow controller found on the PolarisQ, while methanol vapor entered the source by diffusion through an inlet assembly. Because a simple on/off flow regulator opened and closed the methanol CI assembly, the PolarisQ was still capable of performing gas CI.

A TRACE GC Ultra equipped with a pressure-temperature vaporizing inlet (PTV) provided sample introduction. The analytical column is that described in EPA Method 521. Chromatography was optimized for peak shape and compound resolution, and the resulting run time was less than 25 minutes. A cold-splitless injection of 2 µL of sample ensured efficient sample transfer to the column.

The PolarisQ was programmed for MS/MS acquisitions, isolating the precursor for each compound as described in Method 521. Methane was ultimately chosen as reagent gas due to its ionization efficiency and ease of use. Selected method parameters are summarized in Table 2.

TABLE 2. Selected experimental parameters

PolarisQ		TRACE GC Ultra		AS3000 Autosampler	
		Oven Method			
		Initial Temp:	40ºC, hold 2.0 min		
Source Temp:	175 ºC	Ramp 1:	30ºC/min to 60ºC, no hold	Sample Volume (µl):	2.0
Multiplier Offset:	+300 volts	Ramp 2:	5ºC/min to 100ºC, hold 5.0 min	Plunger strokes:	5
Reagent Gas Type:	Methane	Ramp 3:	30ºC/min to 250ºC, hold 4.0 min	Viscous sample:	Yes
Reagent Gas Flow:	1.0 mL/Min	Carrier:	He, constant flow of 1.5 mL/min	Sampling depth in vial:	Bottom
Damping Gas Flow:	0.3 mL/Min	PTV Splitless Method:		Injection depth:	Standard
Max Ion Time:	200 ms	Base Temperature (ºC):	37	Pre-Inj dwell time (sec):	3
Trap Offset: volts	-10.0	Surge:	120 kPa for 2.55 min	Post-Inj dwell time (sec):	3
Emission Current:	250.0 µA	Splitless Duration (min):	1.0		
		Inject Time (min):	0.1		
		Transfer Rate (deg/sec):	14.5		
		Transfer Temperature (ºC):	250		
		Transfer Time (min):	1.0		

FIGURE 4. Calibration curve for NDMA at a 2 µL injection volume, showing linearity from 2-50 ppb. On the right is the extracted ion chromatogram for *m/z* 75, used for quantitation. This shows 10 pg on-column with the 2 µL injection.

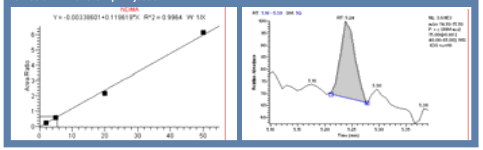


FIGURE 5. Seven replicate injections were performed at the 5 ppb level. Area count precision is shown below for selected compounds.

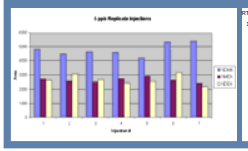
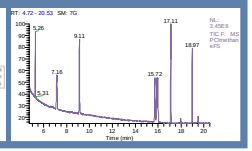


FIGURE 6. Total ion chromatogram of 2ng/µL standard in full scan PCI with methane as reagent gas.



Results

A basic modification of the PolarisQ ion trap allowed a comparison of different reagent gases for performing chemical ionization as applied to the analysis of nitrosamines at drinking water levels. Chromatography was optimized using a 2µL injection volume and a PTV cold splitless injection. Without large volume injection, and with methane as reagent gas, detection limits in the low ppb range were achieved. The extracted ion chromatogram for NDMA at 2 ppb is shown in Figure 4, along with its corresponding calibration curve. The results of the precision study for NDMA at 5 ppb are shown in Figure 5. This shows excellent injection-to-injection reproducibility of the method. Low ppb linearity was also achieved for the remaining compounds in Table 2.

Conclusions

The PolarisQ showed good sensitivity for the analysis of nitrosamines in drinking water. A 2 µL cold splitless temperature programmable injection was made using a narrowbore Silcosteel liner. The PolarisQ, external source ion trap mass spectrometer, was operated in MS/MS mode with CI using methane as the reagent gas. The precision for seven replicate runs of a 5 ppb standard was less than 15 %RSD. The linear regression coefficient was greater than 0.99 for all compounds in the range of 1 to 50 ppb. The peak shape for the nitrosamines was very good. Several reagent gases were evaluated for chemical ionization. Methane was selected because of its strong molecular ion and ease of use in tuning. The mass spectrometer gave very stable response to the target compounds for the duration of the study. Future work will address the optimization of a large volume injection to further lower the detection limit for the method.

References

(1) Mitch, W.A. *et al.* N-Nitrosodimethylamine (NDMA) as a Drinking Water Contaminant: A Review. *Env. Eng. Sci.* **2003**, 20(5), 389-404

(2) Munch, J.W., Bassett, M.V. Method 521: Determination of nitrosamines in drinking water by solid phase extraction and capillary column gas chromatography withy large volume injection and chemical ionization tandem mass spectrometry (MS/MS) (Version 1.0) U.S. Environmental Protection Agency.

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