Analysis of Nine *N*-Nitrosamines Using Liquid Chromatography—High-Resolution, Accurate-Mass Mass Spectrometry

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Highlights

- Sensitive and robust LC-MS method provides for the separation, identification, and quantification of nine *N*-nitrosamines in water.
- Extraction recoveries in drinking water and wastewater matrices ranged from 68% to 83% for eight of the nine target *N*-nitrosamines.
- Detection limits ranged from 0.4 to 12 ng/L.
- LC-MS methodology offers a faster alternative to traditional GC-MS methods for the analysis of nitrosamines.

Introduction

N-nitrosamines present high mutagenic and carcinogenic potential. They or their precursors occur in a wide variety of foods and natural and manufactured products. An analysis method for nine *N*-nitrosamines based on ultra-high performance liquid chromatography combined with high-resolution, accurate-mass (HRAM) mass spectrometry is presented here.

Experimental

Sample Preparation

A primary stock solution of 2000 µg/mL of nine *N*-nitrosamines in methanol was used to prepare working solutions. The internal standard working solutions of *N*-nitrosomethylamine (NDMA-d_6) and *N*-nitrosodipropylamine (NDPA-d_{14}) were prepared from a stock solution containing 1000 µg/mL each. All samples and optimization parameters were done with four replicates (n = 4). For all tests, the sample concentration was set to 5 µg/L and 20 µg/L for the nitrosamines mixture and internal standards mixture, respectively.

Wastewater and drinking water samples were collected from Quebec, Canada. Sample pretreatment and *N*-nitrosamine solid-phase extraction was carried out based on US EPA Method 521.[1] The internal standard mixture solution (NDMA-d_6 and NDPA-d_{14}, 20 µg/L) was added prior to the LC-MS analysis, for quantification.

LC-MS Conditions

UHPLC analysis was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system with a Thermo Scientific™ Hypersil GOLD™ C18 (1.9 µm, 100 × 2.1 mm) column. Mobile phases were (A) water + 0.1% formic acid and (B) methanol + 0.1% formic acid. The injection volume was 100 µL, and the flow rate was 500 µL/min. A Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer was used for analysis.

Scan type	Full scan MS
Resolving power	70,000
AGC	1×10^5
Maximum IT	100 ms
Scan range	*m/z* 50 to 500
Injection volume	100 µL
Spray voltage	5.5 kV
Capillary temperature	350 °C
Heater temperature	100 °C
Sheath gas flow rate	75 Arb
Auxiliary gas flow rate	25 Arb
Ion Sweep gas flow rate	2 Arb
S-Lens RF level	55%
Detection mode	Positive
Lock masses	Off

Thermo Scientific™ Xcalibur™ software version 2.2 SP1 and Thermo Scientific™ Dionex™ DCMSLink ™ software plug-in for Xcalibur software version 2.12 were used for instrument control and data acquisition.

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Data

Table 1. Physicochemical properties, exact mass, and retention time of the studied *N*-nitrosamines. The retention times with the LC-MS method are significantly shorter than those in the GC-MS method.[1]

Compound	Name	Formula	Molecular Mass	Theoretical Precursor $(M+H)^+$	Experimental Precursor $(M+H)^+$	Δ$(M+H)^+$ in ppm	Retention Time (min)
NDMA	*N*-nitrosodimethylamine	$C_2H_6N_2O$	74.04801	75.05584	75.05599	2.0	0.81
NMEA	*N*-nitrosomethylethylamine	$C_3H_8N_2O$	88.06366	89.07149	89.07150	0.1	1.59
NPyr	*N*-nitrosopyrrolidine	$C_4H_8N_2O$	100.06366	101.07149	101.07137	-1.2	1.68
NDEA	*N*-nitrosodiethylamine	$C_4H_{10}N_2O$	102.07931	103.08714	103.08714	0.0	3.34
NPip	*N*-nitrosopiperidine	$C_5H_{10}N_2O$	114.07931	115.08714	115.08697	-1.5	3.47
NMor	*N*-nitrosomorpholine	$C_4H_8N_2O_2$	116.05857	117.06640	117.06612	-2.4	1.12
NDPA	*N*-nitrosodi-n-propylamine	$C_6H_{14}N_2O$	130.11061	131.11844	131.11830	-1.1	3.86
NDBA	*N*-nitrosodi-n-butylamine	$C_8H_{18}N_2O$	158.14191	159.14974	159.14928	-2.9	4.18
NDPhA	*N*-nitrosodi-n-phenylamine	$C_{12}H_{10}N_2O$	198.07931	199.08714	199.08698	-0.8	4.21
NDMA-d_6	*N*-nitrosodimethylamine-d_6	$C_2D_6N_2O$	80.11880	81.10278	81.09364	0.03	0.80
NDPA-d_{14}	*N*-nitrosodi-n-propylamine-d_{14}	$C_6D_{14}N_2O$	144.27439	145.22799	145.20593	0.02	3.85

Figure 1 shows the LC-MS chromatograms of a 20 µg/L nitrosamine standards sample in HPLC-grade water.

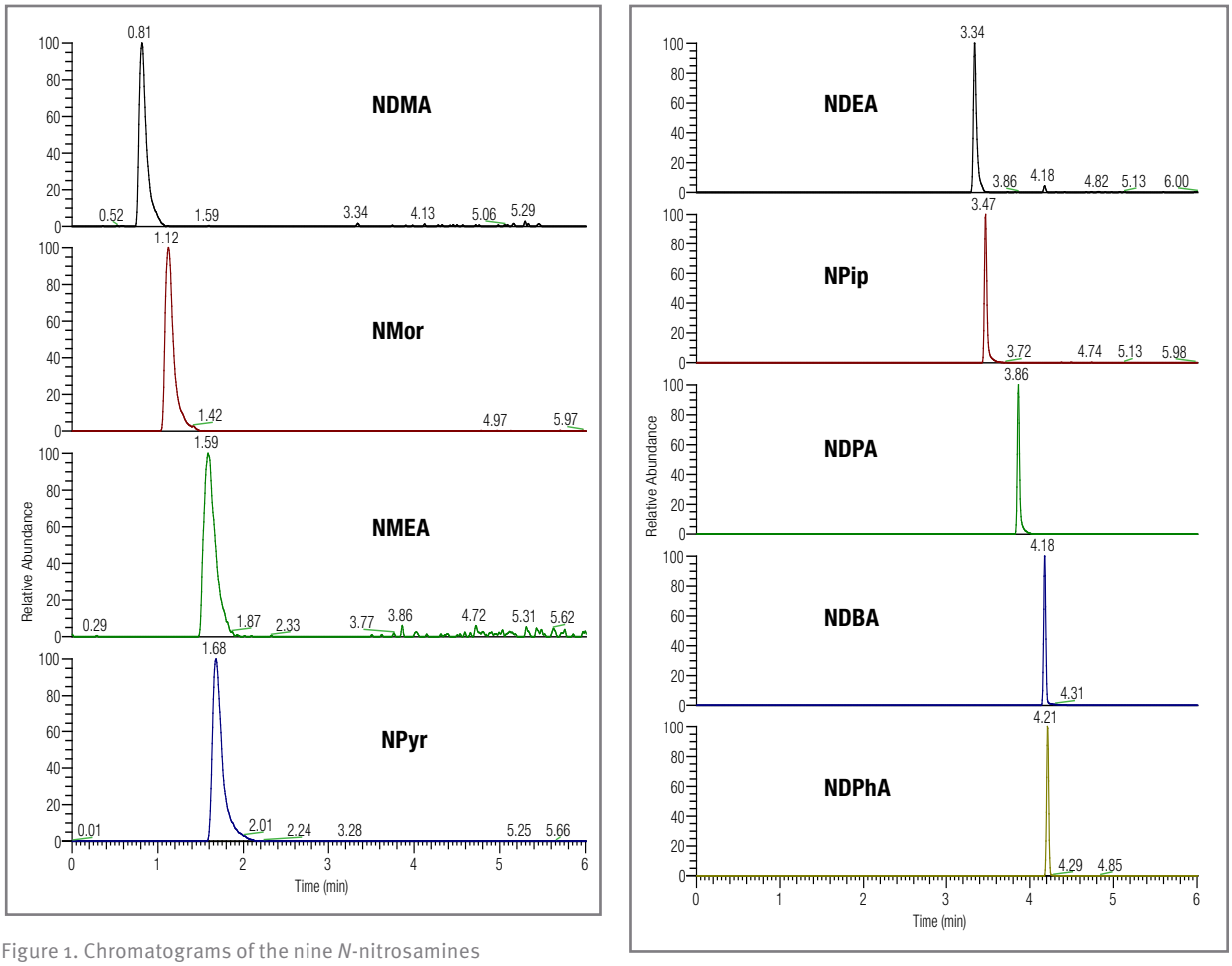

Figure 1. Chromatograms of the nine *N*-nitrosamines

Method Validation

Table 2. Linearity, LOD, and LOQ obtained for the nine analyzed nitrosamines spiked in HPLC-grade water. As no SPE was performed for HPLC-grade water, these values represent the instrumental detection and quantification limits.

Compound	Formula	Linearity range (µg/L)	R^2	LOD (µg/L)	LOQ (µg/L)
NDMA	$C_2H_6N_2O$	0.5–100	0.9996	0.2	0.5
NMEA	$C_3H_8N_2O$	1.0–100	0.9997	0.4	1.0
NPyr	$C_4H_8N_2O$	0.2–100	0.9997	0.05	0.2
NDEA	$C_4H_{10}N_2O$	0.5–100	0.9991	0.15	0.5
NPip	$C_5H_{10}N_2O$	0.05–100	0.9991	0.015	0.05
NMor	$C_4H_8N_2O_2$	0.2–100	0.9997	0.05	0.2
NDPA	$C_6H_{14}N_2O$	0.05–100	0.9996	0.01	0.05
NDBA	$C_8H_{18}N_2O$	0.05–100	0.9969	0.01	0.05
NDPhA	$C_{12}H_{10}N_2O$	0.05–100	0.9939	0.01	0.05

The precision for all nine nitrosamines in HPLC-grade water and the two analyzed water matrices for both QC1 (12 ng/L) and QC2 (120 ng/L) ranged between 0.98% and 19%. The accuracy (% bias) from the expected concentrations, was between 0.09% and 8.2% for HPLC-grade water and between 0.74% and 19% for both types of water matrices. Solid-phase extraction recovery values ranged from 68% to 83%. These values were higher for eight of the nine target NA compared to the overall extraction efficiency of 52% in EPA Method 521.

Table 3. Method validation results for linearity, LOD, and LOQ limits in drinking water and wastewater.

Compound	Drinking water				Wastewater			
	R^2	MLOD (ng/L)	MLOQ (ng/L)	Linearity Range (ng/L)	R^2	MLOD (ng/L)	MLOQ (ng/L)	Linearity Range (ng/L)
NDMA	0.9969	4.2	13	20–200	0.9984	7.6	23	20–200
NMEA	0.9920	9.1	28	20–200	0.9980	12	35	50–200
NPyr	0.9968	1.5	4.6	5–200	0.9975	11	35	50–200
NDEA	0.9955	2.5	7.4	10–200	0.9973	5.9	18	20–200
NPip	0.9973	2.3	7.0	10–200	0.9982	6.4	20	20–200
NMOR	0.9968	6.5	20	20–200	0.9954	4.8	15	20–200
NDPA	0.9961	2.4	7.2	10–200	0.9985	4.7	14	20–200
NDBA	0.9960	1.8	5.3	5–200	0.9972	2.7	8.1	10–200
NDPhA	0.9983	0.4	1.3	1–200	0.9991	2.8	8.4	10–200

Reference

1. Munch, J.W. *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);* U.S. Environmental Protection Agency, Washington DC, 2005; available at http://www.epa.gov/microbes/documents/m_521.pdf.