SI for Methadone Contributes to *N*-nitrosodimethylamine Formation in Surface and Wastewater during Chloramination

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Table SI-1 – NDMA FP recovery after extraction of raw water samples.

	Raw Water NDMA FP (ng)	Reconstituted NDMA FP (ng)	Recovery of NDMA FP (%)	Raw Water DOC (mgC/L)	Raw Water TDN (mgN/L)
SW 1	21 ±0.4	20 ±0.8	95	3.2	0.4
SW 1 (2nd sampling)	22 ±0.7	21 ±0.3	95	3.2	0.4
SW 2	36 ±1.8	29 ±1.2	81	5.3	0.4
SW 3	47 ±0.7	48 ±0.4	102	2.5	2.7
SW 4	97 ±4.6	57 ±3.3	59	6.0	2.7
SW 5	112 ±4.9	71 ±7.2	63	6.4	3.2
SW 6	546 ±3.0	599 ±9.9	110	6.9	4.3
SW7	122 ±9.2	50 ±8.1	41	4.5	4.8
SW8	40 ±2.1	67 ±69.5	168	4.4	0.5
SW9	111 ±2.6	49 ±1.7	44	4.7	4.6
SW10	40 ±1.5	28 ±5.1	70	4.3	0.5
WW 1	602 ±27.5	497 ±25.8	83	5.2	5.4

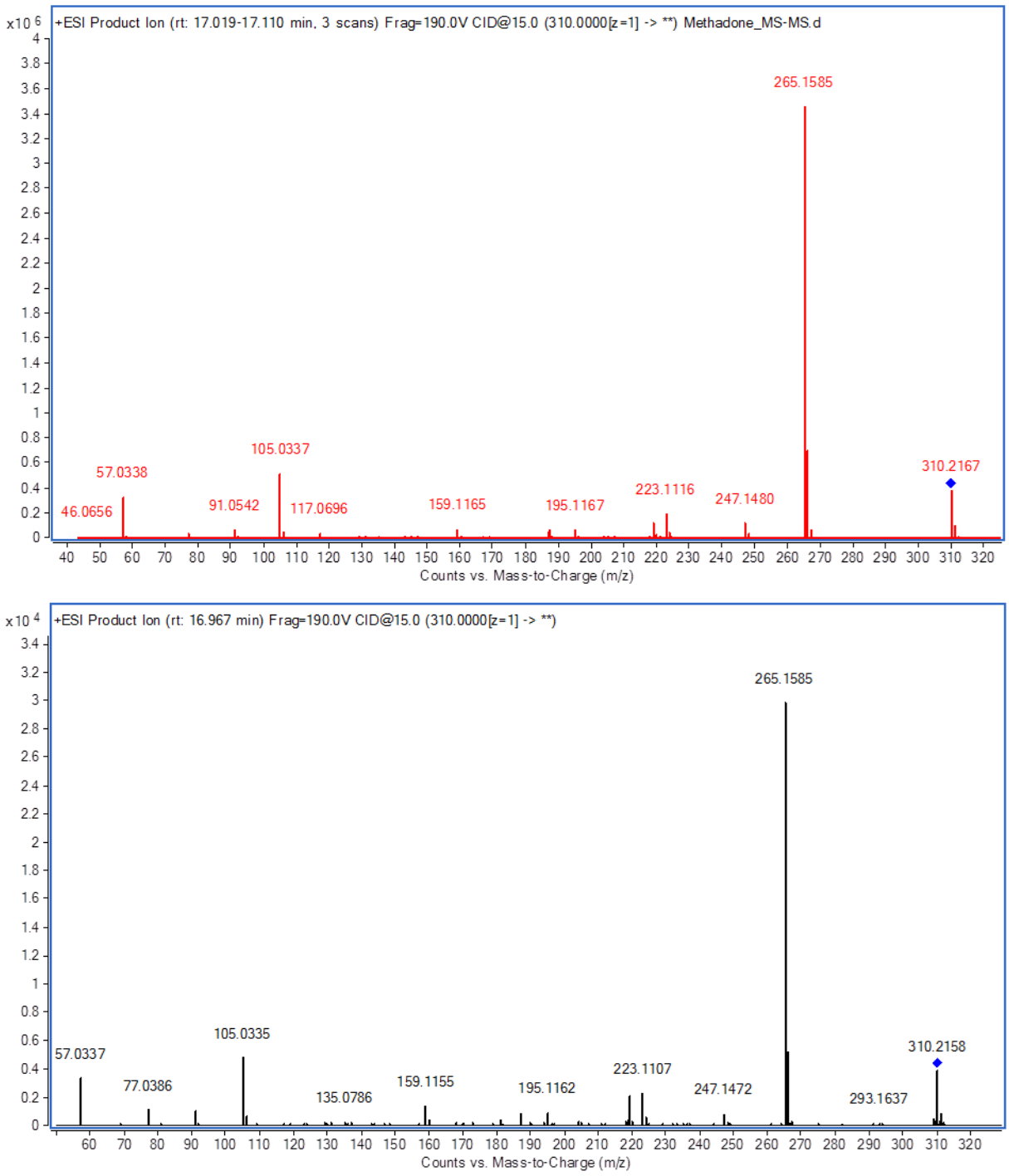

Figure SI-1 – MS/MS spectra of methadone standard (top) and methadone discovered in wastewater (bottom).

1 Reagent Chemicals

Methanol (MeOH) was purchased from Fisher Chemical (Fair Lawn, NJ). Sulfuric acid (H_2SO_4) was purchased from EMD Millipore. Reagent water used was either of HPLC grade (purchased from commercial sources) or was >18.2 MΩ-cm (Megohm-cm). Ascorbic acid, sodium hypochlorite, boric acid, and borax were purchased from Fisher Chemical. Sodium sulfate drying cartridges were from Agilent Technologies (Santa Clara, CA) and methadone, ammonium hydroxide (NH_4OH), formic acid, and sodium hydroxide were from Sigma Aldrich (St. Louis, MO). A NDMA standard was purchased from Supelco (Bellefonte, PA), and the isotopically labeled d6-NDMA standard was from Cambridge Isotopes (Tewksbury, MA). Oasis MCX (500mg) SPE cartridges were purchased from Waters (Milford, MA).

2 NDMA Precursor Isolation

NDMA precursors were concentrated as follows: 3.5 L of filtered sample was pH adjusted to 3 using sequential additions of ≤1M H_2SO_4. 1L of sample was pushed through Oasis MCX SPE cartridges in triplicate at 5 mL/min by an automated SPE system (Caliper Life Sciences Autotrace 280). SPE cartridges were first rinsed with 5 mL MeOH and 5 mL H_2O. After loading, the cartridges were dried for 30 min under a gentle flow of nitrogen gas. Methadone was eluted from the SPE cartridges with 10 mL of 5% NH_4OH in MeOH at a flow rate of 1.5 mL/min. The resulting concentrates were blown down under a gentle stream of nitrogen gas to a final volume of 1 mL (1,000 times concentrated). 500 µL of the extract was reconstituted into Milli-Q™ water and chloraminated under formation potential conditions to quantify bulk precursor recovery. For methadone calculations, we assumed recovery through the cation exchange process was 100% because no labeled isotope of methadone was used and because this results in a likely the most conservative estimate of methadone in the samples.

3 NDMA Formation Tests

Preformed monochloramine was prepared by slowly adding via burette 250 mL of a NaOCl solution to 250 mL solution of 10mM borate buffered (pH 8) NH_4OH solution. The final $N:Cl_2$ ratio was 1.2 and the monochloramine concentration in the stock solution was ~2,000 $mgCl_2/L$ as measured by the indophenol colorimetric Monochlor F method (Hach Company, Loveland, CO).

500 mL sample was buffered with 10mM borate buffer (pH 8), and an appropriate amount of chloramine solution was added. After 72 hours in the dark at 25°C, the chloramines were quenched with 5 mM ascorbic acid, and 200 ng/L of the deuterated NDMA standard was added immediately. Samples were stored for less than two weeks at 4°C in the dark before extraction.

Solid phase extraction followed EPA Method 521.[1] Activated carbon cartridges were washed successively with dichloromethane (DCM), MeOH, and HPLC grade water before being loaded at a rate of 5 mL/min. The columns were dried using compressed nitrogen gas and eluted with 5 mL of DCM. The extract was blown down to 1 mL using a gentle stream of nitrogen gas and transferred to a GC vial via Pasteur pipette. NDMA was quantified by GC/MS (ammonia chemical ionization) using an isotopically labeled standard to quantify losses during solid phase extraction. The details of the NDMA GC/MS method can be found elsewhere.[2]

4 GC/MS Analysis of Methadone

The extract solution was analyzed using Gas Chromatography Mass Spectrometry on an Agilent 6890/5973 inert GC/MS system (Agilent, Santa Clara, CA). The system was equipped with an Agilent HP-5MS column (0.25mm x 30m×0.25µm, Agilent Santa Clara, CA). Helium was used as carrier gas at a constant flow rate of 1.2 mL/min. A generic temperature protocol (i.e., not optimized for methadone) was used in scan mode. In brief, 1 µL of sample was injected. The injector temperature was set at 300°C. The oven temperature started with a hold at 65°C for

10 minutes followed by an increase to 300°C and a final hold for 20 minutes at 300°C. The MS transfer line was set at 275°C, and the MSD was operated in electron impact mode and scanning from m/z 50 to 500 Da. Compound identification and quantification was performed using an authentic methadone standard.

5 LC/Q-TOF/MS Screening Procedure

The separation of the analytes was carried out using an UHPLC system consisting of vacuum degasser, thermostated autosampler, column compartment, and a binary pump (Agilent Series 1290, Agilent Technologies, Santa Clara, CA) equipped with a reverse phase C8 analytical column of 150 mm x 4.6 mm and a 3.5 µm particle size (Zorbax Eclipse XDB-C8). Column temperature was maintained at 25°C. The injected sample volume was 10 µL. Mobile phases A and B were water with 0.1% formic acid and acetonitrile, respectively. The optimized chromatographic method held the initial mobile phase composition (10% B) constant for 5 minutes, followed by a linear gradient to 100% B after 30 minutes. The flow rate used was 0.6 mL/min. A 10-minute post-run was used after each analysis. This UHPLC system was connected to an ultra-high definition quadrupole time-of-flight mass spectrometer model 6540 Agilent (Agilent Technologies, Santa Clara, CA) equipped with electrospray Jet Stream Technology, operating in positive ion mode, using the following operation parameters: capillary voltage 4000 V; nebulizer pressure 45 psig; drying gas 10 L/min; gas temperature 325ºC; sheath gas flow 11 L/min; sheath gas temperature 350ºC; nozzle voltage 1000 V; fragmentor voltage 190 V; skimmer voltage 45 V; and octopole RF 750V. LC/MS accurate mass spectra were recorded across the range 50-1000 m/z at 2 GHz. The data recorded was processed with MassHunter software (version 6.1). Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a low

flow of a calibrating solution (Calibrant Solution A, Agilent Technologies, Inc.), which contains the internal reference masses (purine m/z 121.0509 and HP-921 at m/z 922.0098). The instrument provided a typical mass resolving power of 30,000 at m/z 1522.

6 LC/MS/MS Analysis of Methadone

The separation of methadone in water samples was carried out using an HPLC system consisting of vacuum degasser, autosampler, and a binary pump (Agilent Series 1290, Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed phase C_{18} analytical column of 50 x 2.1 mm and 1.8 µm particle size (Agilent Zorbax Eclipse Plus). Column temperature was maintained at 25°C. The mobile phases A and B were water with 0.1% formic acid and acetonitrile, respectively. Samples were injected (injection volume 15 µL) on to the column. Initial mobile phase composition was 10% B, held constant for 1.7 min, followed by a linear gradient to 100% B at a flow-rate of 0.4 mL/min, for a total run time of 10 min.

The HPLC system was connected to a triple quadrupole mass spectrometer Model 6460 Agilent (Agilent Technologies, Santa Clara, CA, USA) equipped with electrospray Jet Stream technology operating in positive ion mode, using the following operation parameters: capillary voltage 4000 V in positive and 3500V in negative; nebulizer pressure 45 psig; drying gas 10 L/min; gas temperature 250°C; sheath gas flow 11 L/min; sheath gas temperature 350°C; and nozzle voltage 0 V. The fragmentor voltage was 110V, and collision energies were optimized for methadone. Quantitation was performed on the main fragment ion at m/z 265 (collision energy 10V). Two qualifier ions at m/z 105 and 57 were used for unequivocal identification and confirmation of the compound by LC-MS-MS at a collision energy of 20V. The data recorded was processed with MassHunter software (Agilent Technologies).

7 References

1. Munch, J. and Bassett, M., 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). *National Exposure Research Laboratory Office of Research and Development, US Environmental Protection Agency*, **2004**.
2. Hanigan, D., Zhang, J., Herckes, P., Krasner, S. W., Chen, C., and Westerhoff, P., Adsorption of N-Nitrosodimethylamine Precursors by Powdered and Granular Activated Carbon. *Environmental Science & Technology*, **2012**. 46(22): p. 12630-12639.