Finding NDMA Precursors Using Accurate Mass Tools with an Agilent 6540 Q-TOF LC/MS

Application Note

Environment and Food

Authors

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Abstract

N-nitrosodimethylamine (NDMA), a compound of environmental concern, occurs in chloraminated drinking water and treated wastewater effluents. NDMA forms through reactions between chloramines and N-containing organic matter, which for the most part, has not been identified. This application note used accurate mass tools with Q-TOF LC/MS to search and mine for possible precursors of NDMA. This process was accomplished by three approaches using accurate mass. First was the use of a Personal Compound Database Library (PCDL) of known precursors, second was the neutral loss of 45.0578 mass units, which arose from the loss of $(CH_3)_2NH$; this fragment is an indicator of NDMA formation in many model compounds. The third approach was the extraction of the *m/z* 58.0651 ion that forms from the cleavage of the C-R bond β to the dimethylamine nitrogen ($(CH_3)_2NC$-R). The many features of the Agilent MassHunter software were used to carry out these procedures. These accurate mass tools resulted in the identification of a series of pharmaceutical compounds that contribute to NDMA formation in treated drinking water, methadone being an important example. Water samples included drinking water sources and secondary treated wastewater.

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Introduction

N-nitrosodimethylamine (NDMA) is a disinfection by-product that occurs in drinking water. This is an important environmental concern, and is part of the United States Environmental Protection Agency (US EPA) third contaminant candidate list [1]. Canada and two US states (CA, MA) have already set notification or guidance levels in the low 10s of ng/L. During formation of NDMA, one nitrogen atom is derived from the disinfectant, monochloramine [2], and the other N from unidentified sources of organic matter present in the treated drinking water. Treated wastewater discharged to surface waters has been implicated as a source of NDMA precursors because the NDMA formation potential (FP) is 10 to 100 times greater in wastewater than in surface water.

Some precursors of NDMA have been identified, such as polydiallyldimethylammonium chloride (polyDADMAC) (a quaternary amine used in water treatment), ranitidine (a pharmaceutical), dimethylamine (a biological product), and dimethylbenzylamine. All of these compounds have either secondary, tertiary amine, or quaternary structures, which are necessary to form NDMA. Research has followed up on these structures, and searched literature for the occurrence of these compounds in drinking water sources. Because many pharmaceuticals are known to contain dimethylamine structures, and because of their presence in drinking-water sources, researchers have focused on this class of compounds [3], ranitidine being one of the best known examples [4]. Mechanistic studies have also been done [5].

This application note first applied a solid phase extraction (SPE) method that isolated the structural unit based on a cation exchange mechanism, which was then followed by mass spectrometry. The isolation procedure is beyond the scope of this application note, but covered in reference [6]. For mass spectrometry, we used the approach of building a Personal Compound Database Library (PCDL) of known precursors, which can be searched in samples analyzed by Q-TOF LC/MS. This application note shows how to apply the Agilent 6540 Q-TOF LC/MS with Agilent MassHunter software to identify new precursors of NDMA based on fragmentation and formation of diagnostic ions that are related to the dimethylamine structure. To find the 45.0578 mass loss, the neutral loss of the dimethylamine structure was used after application of Auto MS/MS. The approach shown here was modified from our previous publications [7,8], where we examined a number of wastewater and surface water samples in search of NDMA precursors. We show three important accurate mass tools in this application note, which include the:

- Use of the PCDL library of known precursors
- Neutral loss at 45.0578 mass units
- Diagnostic ion approach at *m/z* 58.0578

Experimental

Standards and reagents

Calibration standards were obtained from Cerilliant and AccuStandards at the highest available purity. Calibration standard solutions were prepared in the range of 1–1,000 ng/L. A labeled surrogate internal standard, carbamazepine d-10, was purchased from Cambridge Isotopes. All solvents used were of highest purity available. Pesticide-grade water, methanol, and acetonitrile were obtained from Burdick & Jackson.

Instrumentation

- Agilent 1290
- Agilent 1290 Infinity Binary Pump with internal degasser (G4220A)
- Agilent 6540 Q-TOF LC/MS (G2581-64401)

Concentrated precursors to NDMA were analyzed with an Agilent 1290 Infinity UHPLC with a C8 analytical column (Agilent ZORBAX Eclipse XDB-C8) coupled to an Agilent 6540 Ultra-high Definition Quadrupole Time-of-Flight Mass Spectrometer (Q-TOF LC/MS) controlled using Agilent MassHunter v6.01.

Table 1 shows the analytical operating conditions for the Agilent 6540 Q-TOF LC/MS system.

Table 1. HPLC and LC/QTOF-MS Conditions

HPLC conditions			
Analytical column	Agilent ZORBAX Eclipse XDB C8, 150 × 4.6 mm, 3.5 µm (p/n 963967-906)		
Temperature	25° C		
Mobile phase	A) Water (0.1% acetic acid) B) Acetonitrile		
Flow rate	0.6 mL/min		
Gradient for elution from the column	Time (min)	%A	%B
	0	90	10
	5	90	10
	30	0	100
MS and MS-MS conditions			
Acquisition parameters	Q-TOF LC/MS Positive Ion		
Sheath gas temperature	350 °C		
Sheath gas flow rate	11 L/min		
Drying gas temperature	250 °C		
Drying gas flow rate	10 L/min		
Nebulizer pressure	45 psig		
Nozzle voltage	0 V		
Vcap	3,500 V		
Fragmentor	190 V		
Skimmer	65 V		
Oct 1 RFpp	750 V		
Precursor ion selection	Static exclusion 100–130 and 500–1,000 10,000 abundance at 0.01 % Spectra acquisition rate: 4 spectra/second Pulsed at 100–3,000 mass range for MS Pulsed at 100–3,000 mass range for MS/MS MS and MS/MS Range at 2 GHz		
Collision energy	15 V		
Preferred list	None		

Sample preparation

Surface water and wastewater samples were taken by our colleagues and by us at two locations, the South Platte River near Denver, Colorado, and the Mesa wastewater sample from Phoenix, Arizona. The South Platte sample is a drinking water source in Denver, CO and other municipalities along the 600-mile flow path to the Missouri River. The sample was prepared by SPE, as explained in reference [6], using cation-exchange to capture basic amine molecules. The cation-exchange SPE was loaded at pH 3, and eluted using 5 % NH_4OH in methanol, which was partially evaporated to remove ammonia. Extracts were diluted 1/10, and 20 µL were analyzed by LC/Q-TOF-MS.

Results and Discussion

Suspect/nontarget analysis with PCDL

To search the extracts of water samples analyzed by Q-TOF LC/MS (Table 2), a PCDL was developed for 66 known or suspected precursors of NDMA. The L portion of the PCDL consists of a table of compounds, their formulas, and exact masses, and is an important tool in MassHunter. If retention times exist in the PCDL, they may also be used along with the accurate mass in, for example, a 5-ppm mass window. When either MS/MS or All Ions is used, the PCDL has a greater confidence of correct identification because fragment ions are present. This database was applied to the surface water and wastewater samples where we found detections of the NDMA precursors: metformin, DEET (N-nitrosodiethylamine [NDEA] precursor), diuron, diphenhydramine, tramadol, doxylamine, venlafaxine, diltiazem, azithromycin, ranitidine, and roxithromycin. The combined NDMA formation potential (FP) for these detections account for a total of 50 ng/L, less than 10 % of the NDMA FP of the raw water [7,8].

Because this is still a small component of the total formation potential, the second tool, which is the diagnostic fragmentation patterns of known NDMA precursors, was tested.

Table 2. PCD of 66 Known or Suspected Precursors of NDMA

Compound	Formula	Neutral mass
Dimethylamine	C_2H_7N	45.05785
Trimethylamine	C_3H_9N	59.0735
Dimethylformamide (DMFA)	C_3H_7NO	73.05276
N,N-Dimethylethylamine	$C_4H_{11}N$	73.08915
Tetramethylamine	$C_4H_{12}N$	74.09697
Dimethylaminoacetonitrile	$C_4H_8N_2$	84.06875
N,N-Dimethylisopropylamine	$C_5H_{13}N$	87.1048
Dimethylethylenediamine	$C_4H_{12}N_2$	88.1000
Dimethylaminoethanol	$C_4H_{11}NO$	89.0840
Dithiocarbamate	CH_3NS_2	92.97069
N,N-Dimethylbuthylamine	$C_6H_{15}N$	101.1205
Dimethyltert-butylamine	$C_6H_{15}N$	101.1205
Choline	$C_5H_{14}NO$	104.1075
2-Dimethylaminoethanethiol	$C_4H_{11}NS$	105.0612
Dimethyldithiocarbamate	$C_3H_7NS_2$	121.0020
N,N-Dimethylaniline	$C_8H_{11}N$	121.0892
4-Dimethylaminopyridine	$C_7H_{10}N_2$	122.0844
2-Dimethylaminopyridine	$C_7H_{10}N_2$	122.0844
Metformin	$C_4H_{11}N_5$	129.1015
N,N-Dimethylbenzylamine	$C_9H_{13}N$	135.1048
3-Dimethylaminophenol	$C_8H_{11}NO$	137.0841
Dimethylthiophene-2-methylamine	$C_7H_{11}NS$	141.0612
Dimethylphenethylamine	$C_{10}H_{15}N$	149.1205
2-Chloro-N,N-dimethylaniline	$C_8H_{10}ClN$	155.0502
Dimethylaminomethylfurfurylalcohol	$C_8H_{13}NO_2$	155.0946
3-(Dimethylaminomethyl)indole (DMAI)	$C_{11}H_{14}N_2$	174.1157
DEET	$C_{12}H_{17}NO$	191.1310
Isoproturon	$C_{12}H_{18}N_2O$	206.1419
N,N-dimethyldodecan-1-amine oxide	$C_{14}H_{31}NO$	229.2406
4-dimethylaminoantipyrine (DMAP)	$C_{13}H_{17}N_3O$	231.1372
Diuron	$C_9H_{10}Cl_2N_2O$	232.0170
Lidocaine	$C_{14}H_{22}N_2O$	234.1732
Thiram	$C_6H_{12}N_2S_4$	239.9883

Compound	Formula	Neutral mass
Tetrabutylamine	$C_{16}H_{36}N$	242.2848
Cocamide mea	$C_{14}H_{29}NO_2$	243.2198
Diphenhydramine	$C_{17}H_{21}NO$	255.1623
Cetyltrimethylamine	$C_{17}H_{38}N$	256.3004
Tramadol	$C_{16}H_{25}NO_2$	263.1885
Des-venlafaxine	$C_{16}H_{25}NO_2$	263.1885
Auramine	$C_{17}H_{21}N_3$	267.1736
Doxylamine	$C_{17}H_{22}N_2O$	270.1732
Chlorphenamine	$C_{16}H_{19}ClN_2$	274.1237
Benzyldimethyldodecylamine	$C_{19}H_{34}N$	276.2691
Benzyltributylamine	$C_{19}H_{34}N$	276.2691
Amitriptyline	$C_{20}H_{23}N$	277.1831
Venlafaxine	$C_{17}H_{27}NO_2$	277.2042
Doxepin	$C_{19}H_{21}NO$	279.1623
3-N,N-DAPSIS	$C_{13}H_{29}NO_3S$	279.1868
Methylene blue	$C_{16}H_{18}N_3S$	284.1221
Carbinoxamine	$C_{16}H_{19}ClN_2O$	290.1186
Sumatriptan	$C_{14}H_{21}N_3O_2S$	295.1355
Benzalkonium chloride	$C_{21}H_{38}N$	304.3004
Methyl Orange	$C_{14}H_{15}N_3O_3S$	305.0834
Ranitidine	$C_{13}H_{22}N_4O_3S$	314.1413
Citalopram	$C_{20}H_{21}FN_2O$	324.1638
Nizatidine	$C_{12}H_{21}N_5O_2S_2$	331.1137
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	335.1093
Cocamidopropyl betaine	$C_{19}H_{39}N_2O_3$	343.2961
Methyl violet B	$C_{25}H_{30}N_3$	372.2440
Diltiazem	$C_{22}H_{26}N_2O_4S$	414.1613
Mifepristone	$C_{29}H_{35}NO_2$	429.2668
Tetracycline	$C_{22}H_{24}N_2O_8$	444.1533
Minocycline	$C_{23}H_{27}N_3O_7$	457.1849
Clarithromycin	$C_{38}H_{69}NO_{13}$	747.4769
Azithromycin	$C_{38}H_{72}N_2O_{12}$	748.5085
Roxithromycin	$C_{41}H_{76}N_2O_{15}$	836.5246

Diagnostic fragmentation patterns of known NDMA precursors

Based on literature, tertiary amines, with dimethylamine substituents, typically have greater NDMA yield than secondary, primary, or quaternary amines [3-5]. Twenty-five known nitrosamine precursors with the dimethylamine structure were analyzed by Q-TOF LC/MS/MS to determine their fragmentation patterns. The purpose of this experiment was to find common diagnostic ions or common neutral losses that could be used to find new precursors of NDMA. In particular, the focus was on the dimethylamine structure and if it could be recognized by either a diagnostic ion fragment or by a neutral loss using MS/MS analysis. Figure 1 shows the mass spectrum for two known NDMA precursors, dimethylphenethylamine and tramadol.

Note that both dimethylphenethylamine and tramadol have the tertiary amine structure. However, in one case, the major diagnostic ion is the *m/z* 58.0651 (tramadol), while the other example, dimethylphenethylamine, gives a neutral loss of 45.0578 mass units with only trace amounts of the *m/z* 58.0651 ion. In both cases, either the ion or the neutral loss is indicative of the characteristic structure that we were looking for: the tertiary amine that consists of the dimethyl group. This is important in that the formation of NDMA is derived from the dimethyl amine moiety, as shown in Figure 2, with water supplying the oxygen atom needed.

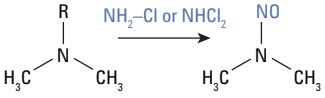


Figure 2. Formation of NDMA from a precursor structure using chloramine as the N source.

Figure 3 shows the MS/MS results for 21 compounds. Each compound is shown to have either the absence or presence of diagnostic fragments. The neutral loss of 45.0578 mass units arises from cleavage of the $(CH_3)_2N$ bond at the amine nitrogen (Figure 2), while the 58.0651 *m/z* ion likely arises from the cleavage at the $(CH_3)_3N$ bond β to the amine nitrogen. From the list of compounds shown in Figure 3, the compounds with the highest NDMA formation potential are in the top, showing only the 45.0578 mass unit neutral loss. This is an important insight that was used to search for unknown NMDA precursors in the following section.

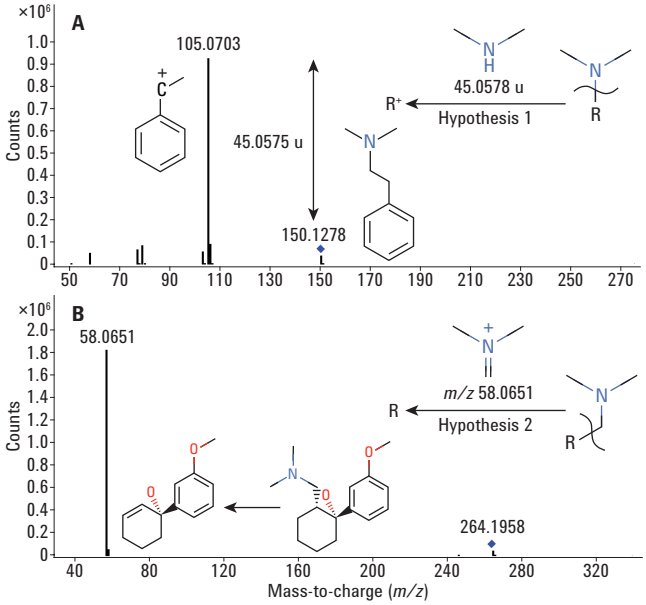


Figure 1. Q-TOF LC/MS/MS spectra of dimethylphenethylamine (A) and tramadol (B). Copyright 2016, reproduced with permission of Elsevier [7,8].

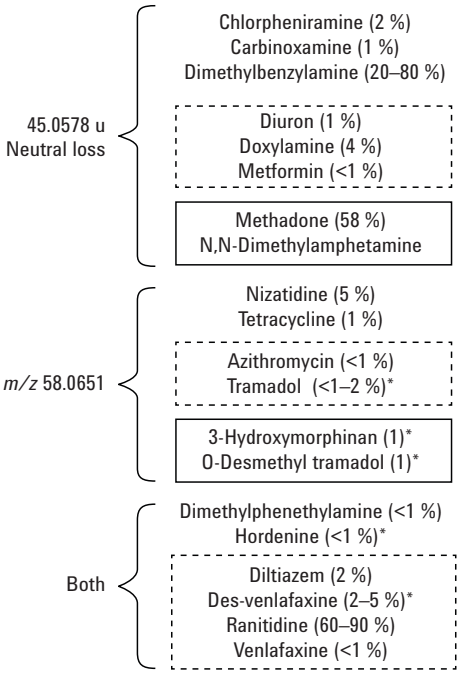


Figure 3. The results for compounds tested with neutral loss, diagnostic ion m/z 58.0561, and those compounds containing both the diagnostic ion and the neutral loss. No box: NDMA precursors used to develop the two diagnostic fragmentation hypotheses, but with no occurrence in samples in this study. Dashed boxes: precursors used to develop the fragmentation hypotheses, later found in water samples. Solid boxes: precursors found in water samples using the diagnostic fragmentation patterns. Copyright 2016, reproduced with permission of Elsevier [7,8].

There were several compounds that did not fragment to either the 45.0578 neutral loss or the *m/z* 58.0561 ion. Only one has a reported yield above 1 %, which suggests that these two fragmentation pathways are critical to NDMA formation, and are useful diagnostic tools in the search for NDMA precursors.

Finding NDMA precursors using accurate mass tools

Using the two diagnostic fragmentation patterns specific to NDMA precursors, we searched for these patterns in two water samples, the Mesa wastewater and the South Platte River. The procedure we initially used involved the development of a MatLab script to look for neutral losses of 45.0578. This procedure resulted in a discovery of methadone as an important precursor of NDMA that was not known prior to our research [5,6]. Subsequently, we applied the neutral loss feature of the MassHunter software, to find new neutral losses consistent with the formation of NDMA. To use this feature, run the sample in Auto MS/MS with the following settings:

1. Go to the MassHunter software and tab Find Compounds.
2. Find Auto MS/MS, and click the Processing tab.
3. Enter a neutral loss of 45.0578 with a tolerance of 0.005.
4. Analyze the sample by Auto MS/MS mode since this gives rise to a series of fragment ions that can be associated with a neutral loss of 45.0578.

Figure 4 shows the results of the neutral loss experiment for the wastewater sample called Mesa, from Mesa, Arizona. There were 20 major losses of 45.0578 from this experiment, as set by the software. One can choose the number of losses by intensity. The ion that resulted in the 45.0578 loss was the *m/z* 310.2152 ion, with the base peak ion at 265.1577. This loss of the dimethylamine group suggests a stable base peak at *m/z* 265.1577, and a fragile precursor ion at *m/z* 310.2152. The fact that the precursor ion had experienced a 45.0578 loss was an important indicator, and we hypothesized that this neutral loss would give rise to a large NDMA formation potential. The compound was previously identified as methadone [5,6]. We discuss this identification in a following section.

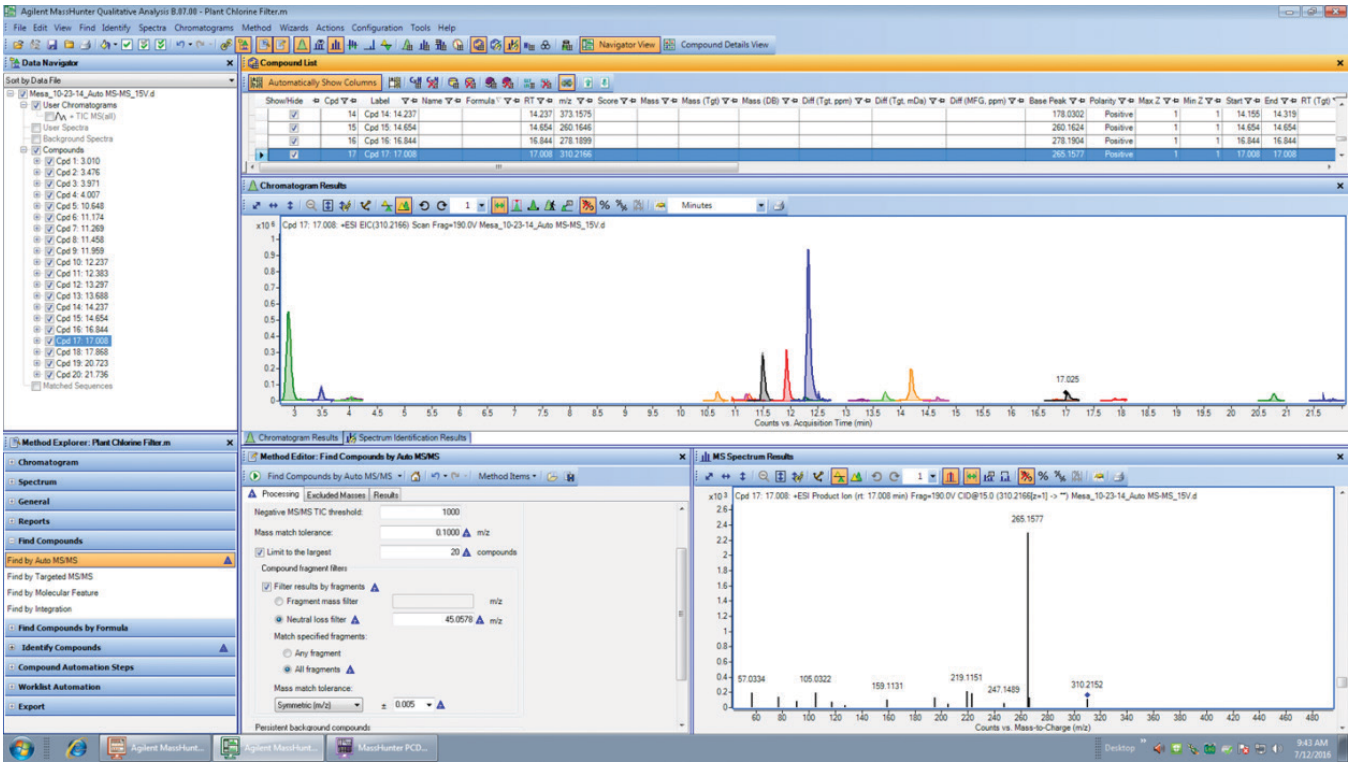
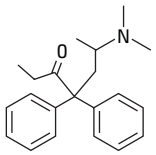


Figure 4. Results of neutral loss (45.0578) search for a compound with a mass of 310.2152.

We also searched for the diagnostic *m/z* 58.0561 fragment ion using the All Ions feature in the MassHunter software. For example, in Figure 5A, we show the extracted ion chromatogram for the *m/z* 58.0651 ion for the Mesa wastewater, and in Figure 5B the same extracted ion for the South Platte River sample. The use of the All Ions feature in the MassHunter software clearly made a difference in the intensity of the *m/z* 58.0651 ion. The South Platte River sample did not use All Ions, and it matches the Mesa sample quite closely. This is due to the fact that the South Platte River receives up to 100 million gallons of wastewater on a daily basis from the Denver Metro wastewater treatment plant. This source impacts the South Platte River for hundreds of miles until dilution occurs from the North Platte River in Nebraska. It appears that a collision energy of 10 V is a good setting to generate the maximum signal for this NDMA diagnostic ion.

Identification of NDMA precursors by Q-TOF LC/MS and MS/MS analysis

Once a likely precursor candidate was identified by either of the diagnostic fragmentation patterns, we proposed an elemental composition (molecular formula) based on the accurate mass of the parent ion using the Identify Compounds and Generate Formula tabs in the MassHunter software. An example is the neutral loss for the *m/z* 310.2152 ion shown in Figure 4. A formula was generated for this ion of $C_{21}H_{27}NO$. Chemspider was searched, and over 1,000 compounds matched this formula. However, the formulae may be ranked by the number of references, which gave several possibilities. When the structure necessary to form NDMA was also used to screen the possible hits, the list shrank to only one, methadone, a drug used to treat heroin addiction, and a likely target to be found in wastewater associated with large cities. Figure 6 shows the structure for methadone, with the dimethylamine moiety.



Methadone

Figure 6. Chemical structure of methadone.

Figure 5. A) Extraction of the m/z 58.0651 ion at a +0.01 mass units for the Mesa sample using All Ions with possible compounds. (B) South Platte River sample with extraction of the m/z 58.0651 ion.

Finally, we carried out an MS/MS experiment, as shown in Figure 7, and, in this case, matched the MS/MS spectrum to the one in the Agilent Forensic Toxicology PCDL library for methadone. Verification was completed with the analysis of a standard. We then did NDMA formation potentials and found that methadone was able to give up to 58 % molar conversion to NDMA [5].

We moved on to quantify methadone in the samples, and determined the contribution to the total NDMA formation. Methadone contributed from 1 to 10 % of the total NDMA formation potential in surface waters, and up to 62 % in wastewater [5,6].

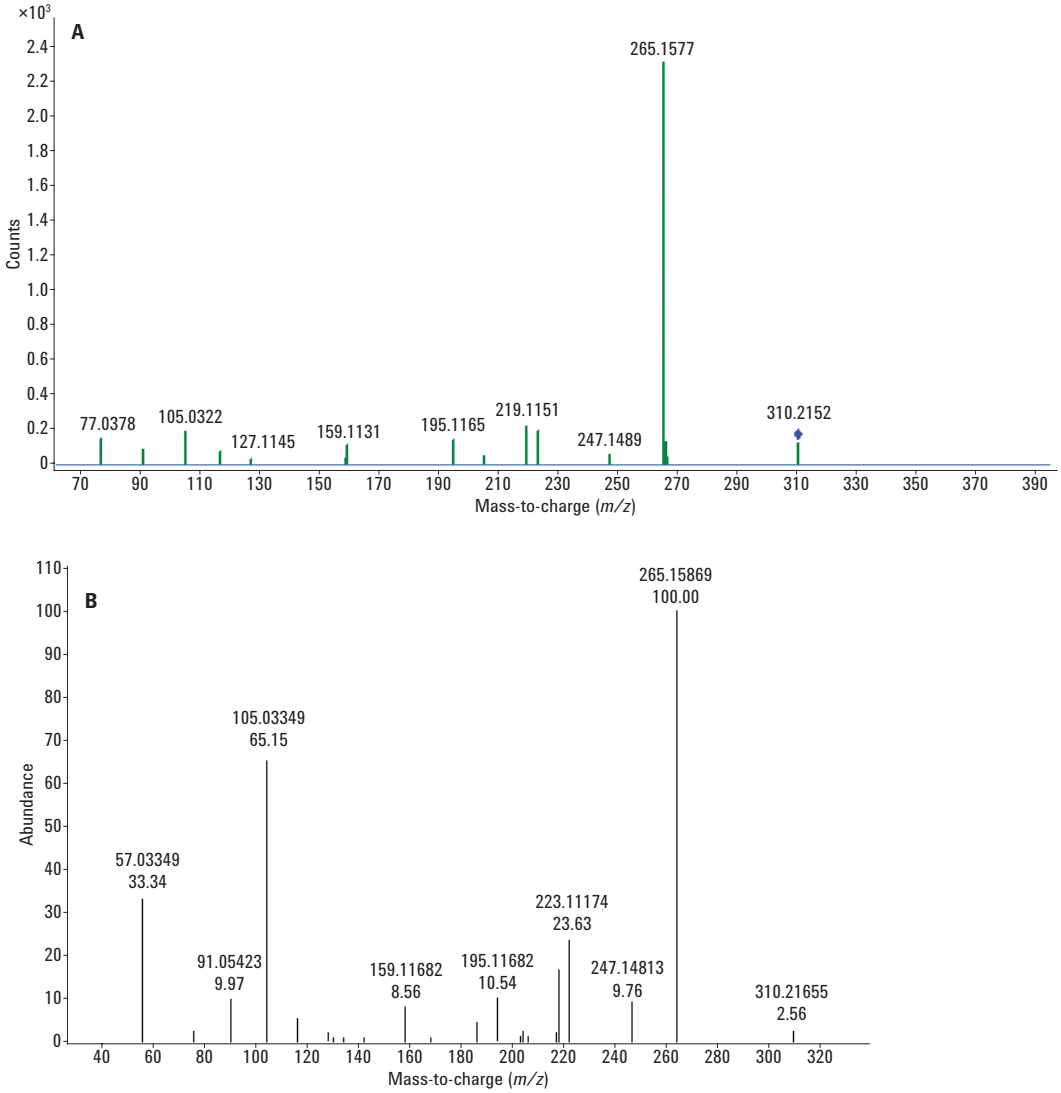


Figure 7. MS/MS identification of 310 ion, where A is the measured spectrum and B is the library analysis of methadone in the Agilent Forensic Toxicology PCDL library.

Conclusion

Using Agilent accurate mass tools, we identified methadone and other NDMA-forming compounds in surface water and wastewater samples. See references [7,8] for further reading.

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Acknowledgements

The authors gratefully acknowledge the support received from participating utilities and from the funding sources: The Water Research Foundation (Project #4499 managed by Djanette Khiari).

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Printed in the USA
May 2, 2017
5991-7730EN

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