IDENTIFICATION OF POLAR DRINKING WATER DISINFECTION BY-PRODUCTS WITH LC/MS

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Abstract. Alternative disinfectants, such as ozone, are gaining in popularity due to stricter regulations on chlorination by-products, namely trihalomethanes (THMs) and haloacetic acids (HAAs). However, there is still much not known about the identity of disinfection by-products (DBPs) from alternative disinfectants, such as ozone. And, therefore, it is not known if these alternatives form DBPs that are more or less harmful than those of chlorine. Because it is currently believed that many of the previously unidentified DBPs are polar in nature, and hence, difficult to extract from water and identify, we have developed a method that can be used to identify polar DBPs. This method involves the use of 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by analysis with liquid chromatography (LC)/negative ion-electrospray mass spectrometry (MS), and will allow the identification of polar aldehydes and ketones in drinking water. This method offers advantages over the currently accepted method using pentafluorobenzylhydroxylamine (PFBHA) derivatization and gas chromatography (GC)/MS analysis. This DNPH-LC/MS method allows for the detection of highly polar carbonyl compounds (with multiple polar substituents) and produces mass spectra and chromatographic behavior that can be used to distinguish between aldehydes and ketones in ozonated water. Using this method, we have successfully analyzed many polar-substituted aldehyde and ketone standards and have identified new DBPs from ozone that have not been previously reported.

INTRODUCTION

Although chlorine has been used to disinfect drinking water for approximately 100 years, there have been concerns raised over its use, due to the formation of some potentially hazardous by-products. Because of these concerns, alternative disinfectants are being explored. Ozone is one of the most popular alternatives, as it is effective against resistant microorganisms and it does not form the chlorine-containing by-products that are of concern. However, there is still much not known about the DBPs formed by ozone. Compounds that have been identified to date include aldehydes, ketones, and carboxylic acids. The major uncertainty relative to ozone DBPs is the polar by-products that are believed to be present, but have not been identified due to the difficulty in extracting them from water.

We have recently developed a method using derivatization with DNPH, followed by analysis using negative ion electrospray LC/MS. Using this new method, we have been able to analyze polar carbonyl compounds, and we have identified ozone drinking water DBPs that have never been reported. In addition, with this method, aldehydes and ketones can be easily distinguished--which is difficult, and sometimes impossible, using existing GC/MS methods.

EXPERIMENTAL

Ozonated water was collected from four sources: 1) a full-scale ozone treatment plant in Valdosta, GA, 2) a full-scale ozone treatment plant in Gwinnett County, GA (metropolitan Atlanta), 3) a pilot ozonation plant in Jefferson Parish, LA, and 4) laboratory-scale ozonations carried out on Suwannee River humic and fulvic acid-fortified distilled water (5 mg/L), with an ozone dose of 2:1 ozone:dissolved organic carbon (DOC).

DNPH derivatizations were carried out using a method similar to that published by Grosjean and Grosjean (Grosjean and Grosjean, 1995).

LC/MS analyses were accomplished using a Hewlett Packard 1050 LC coupled with a Fisons Platform quadrupole mass spectrometer. Electrospray ionization was used with a flow rate of 0.3 mL /min and a source

temperature of 160 °C. Scans were performed over a mass range of 30 to 700 Da, and the cone voltage was alternated between 15V and 30V to alternately obtain mass spectra containing mostly unfragmented molecular ions (15V) and collisionally induced fragmentation (CID) spectra (30V). Samples (50 μL) were injected onto a Supelco Supelcosil C18 LC column (5 μm particle size, 150 x 2.1 mm i.d.), which was eluted using a gradient of 50:50 acetonitrile/water to 98:2 acetonitrile/water over 60 min.

RESULTS AND DISCUSSION

Derivatization with a pre-charged derivatizing agent is often used by LC/MS researchers to enhance the signal of charged ions (usually positive). However, when attempting to identify parts-per-billion (ppb) levels of drinking water DBPs in a complex mixture, we found this approach did not work well. Once formed, the pre-charged derivatives could not be easily separated from salts, were difficult to concentrate, and were not easily separable by LC. The presence of salts enables the formation of confusing adduct ions (e.g., $[M+Na]^+$, $[M+Ca]^+$) in the mass spectrometer, and without concentration of the sample, MS signals are not of sufficient quality to allow unknown identifications to be made. As a consequence, we used a different approach, whereby derivatives are not pre-charged, but can be readily charged in the electrospray interface.

Derivatization with DNPH accomplishes this. Figure 1 illustrates the DNPH reaction. Similar to the PFBHA reaction, the NH_2 group of DNPH reacts with carbonyl groups, forming a hydrazone. Because the derivative is not pre-charged, it can be easily concentrated onto an ordinary C18 cartridge. This allows for improved detection and for any salts to be removed from the sample (salts are unretained on the C18 phase). However, because the two nitro groups render the NH group acidic, negative ions are formed with abundance with electrospray-MS, allowing for good detection of drinking water DBPs.

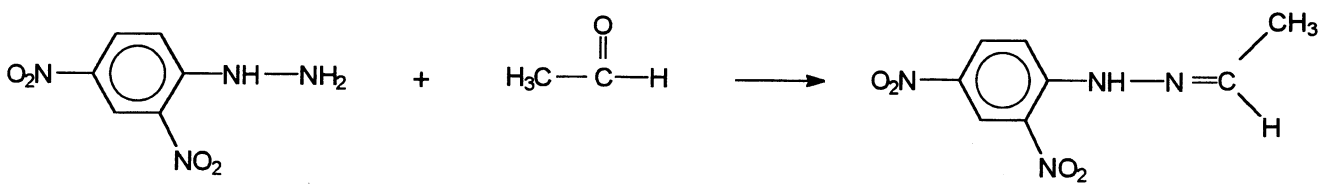
To optimize chromatographic and MS conditions, a mixture of aldehydes, including known ozone DBPs, was used. Chromatographic conditions that worked best for separating the aldehydes (ranging from formaldehyde to decanal) involved gradient elution of 50:50 water/acetonitrile to 2:98 water/acetonitrile over 60 min. Di-aldehydes and keto-aldehydes also worked well by this method. Unlike GC/MS chromatograms, where chemical peaks are easily recognizable, we found it necessary to display reconstructed ion chromatograms (from the specific mass of the chemical ion of interest) in order to find the analytes. A series of ketones, with the carbonyl group on carbon-2 (ranging from acetone to 2-decanone) were also well separated using these chromatographic conditions. The aldehydes and ketones tested by this method are shown in Table 1.

Next, a series of polar aldehydes and ketones was derivatized and analyzed using this method. These compounds included hydroxy-aldehydes, hydroxy-ketones, aldo-acids, and keto-acids (Table 1). These compounds do not work well by the PFBHA-GC/MS method, but worked well with this DNPH-LC/MS method. Molecular ions that were not evident by GC/MS (electron ionization) were clearly evident in the LC-electrospray mass spectra, and compounds that were not evident at all by GC/MS (due to poor chromatography) were clearly visible by LC/MS.

Each of the derivatized aldehydes and ketones we studied shared some common fragments that were observed in the mass spectra obtained using in-source collisionally induced dissociation (CID) at a cone voltage of 30 V. These common fragments included $(NO_2)^-$, $(M-NO)^-$, and $(M-HNO_2)^-$. Because these ions are consistent, neutral loss scans for these ions could be used to selectively detect the DNPH derivatives, eliminating much of the chemical noise background.

Distinguishing Aldehydes from Ketones

In addition to improving the detection and identification of polar ozone by-products, this DNPH LC/MS method can also be used to distinguish aldehydes from ketones. Using the PFBHA-GC/MS procedure,



DNPH Acetaldehyde Hydrazone Derivative

Figure 1. DNPH derivatization.

Table 1. Examples of Compounds That Worked Well by DNPH-LC/MS Method

Compound	MW	Derivatized MW $(M-H)^-$
Aldehydes		
*Formaldehyde	30	209
*Acetaldehyde	44	223
*Propanal	58	237
*Butanal	72	251
*Pentana	86	265
*Hexana	100	279
*Heptan	114	293
*Octana	128	307
*Nonanal	142	321
*Decanal	156	335
Ketones		
*Acetone	58	237
*2-Butanone	72	251
*2-Pentanone	86	265
*2-Hexanone	100	279
2-Heptanone	114	293
2-Octanone	128	307
2-Nonanone	142	321
2-Decanone	156	335
Di-aldehydes		
*Glyoxal	58	417
Keto-aldehydes		
*Methylglyoxal (2-ketopropanal)	72	431
*5-Ketohexanal	114	473
Hydroxy-aldehydes		
2-Hydroxybutanal	88	267
3-Hydroxybutanal	88	267
4-Hydroxybutanal	88	267
2,5-Dihydroxybenzaldehyde	138	317
2,2-Dimethyl-3-hydro-xypropionaldehyde	102	281
Hydroxy-ketones-		
3-Hydroxy-2-butanone	88	267
5-Hydroxy-2-pentanone	102	281
4-Hydroxy-4-methyl-2-pentanone	116	295
*6-Hydroxy-2-hexanone	116	295
*1,3-Dihydroxyacetone	90	269
Aldo-acids		
*Glyoxylic acid	74	253
Keto-acids		
*Pyruvic acid (2-ketopropanoic acid)	88	267
5-Ketohexanoic acid (4-acetylbutyric acid)	130	309
*Ketomalonic acid (2-ketopropanedioic acid)	118	297

* Denotes a compound identified in an actual ozonated water sample

aldehydes and ketones cannot be clearly distinguished from their EI spectra, as they both form mostly the same ions, with some minor differences in relative abundances. With negative ion electrospray MS, using CID at a cone voltage of 30V, however, we found a unique ion (m/z 163) that was formed only for aldehydes and not for ketones (as observed for a series of C3 to C10 aldehydes and ketones, also for those with hydroxy substituents). This fragment ion was attributed to fragmentation of the $CH\text{-}CH_2$ bond of the aldehyde (Figure 2) along with a loss of a NO_2 group, and would not be possible for ketones.

Aldehydes and ketones can also be distinguished by their LC chromatographic peaks. In the DNPH reaction, two isomers--*syn* and *anti*--are formed. Using the chromatographic conditions reported here, ketones show 2 chromatographic peaks for the *syn* and *anti* isomers, whereas the aldehydes show only one peak. Both *syn* and *anti* isomers are formed for the aldehydes, but they co-elute using these chromatographic conditions. The mass spectra were identical for these isomers, and therefore, could not be used to distinguish between them. This observed difference in chromatographic behavior, however, can be used to readily distinguish aldehydes from ketones. We found that not only do straight chain aldehydes and ketones show this chromatographic behavior, but also hydroxy-branched aldehydes and ketones exhibit it, as well.

Identification of New DBPs

Compounds that were found to be DBPs in samples of ozonated drinking water (either from full-scale plants or from our laboratory ozonations of humic acid) are indicated with asterisks in Table 1. Several of these compounds have not been previously reported, including 1,3-dihydroxyacetone, 6-hydroxy-2-hexanone, and 5-ketohexanal.

LITERATURE CITED

Grosjean, E. and Grosjean, D., 1995. Liquid chromatographic analysis of C_1-C_{10} carbonyls. *Int. J. Environ. Anal. Chem.* 61:47-64.