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Development of a Method to Identify Keto Acids in Ozonated Fulvic Acid Solutions

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A gas chromatographic-mass spectrometric method, combining an *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine–diazomethane double derivatization, was developed to identify major keto acids in ozonated fulvic acid solutions. Three keto acids, glyoxylic, pyruvic, and ketomalonic acid, four aldehydes and two analytical artifacts were identified. The detailed mass spectra, obtained in both electron impact and positive chemical ionization modes, and their fragment assignments are presented. Owing to the similarity of their electron impact mass spectra, electron impact mass spectra along with positive chemical ionization mass spectra are needed to identify these keto acids reliably. The effects of the formation of keto acids on water quality are also discussed.

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INTRODUCTION

Since the 1970s, a number of disinfection byproducts (DBPs) have been identified in chlorinated drinking waters. These DBPs include trihalomethanes, haloacetic acids, trihaloacetaldehydes, cyanogen halides, haloacetones, haloacetonitriles and MX.[1–7] Because of concerns over the health effects of these compounds, the US Environmental Protection Agency has proposed new DBP regulations in the coming disinfectants–disinfection byproducts (D-DBP) rule.[8] Ozonation has been used in many water utilities in North America as a treatment technique for controlling DBPs.[9] Like chlorine, ozone also reacts with natural organic matter in water to produce various organic byproducts. Compared with chlorinated DBPs, however, very little information is available on the ozonation byproducts.[10] This has been partly attributed to the difficulty in quantifying and identifying these compounds. Analytical difficulties stem from the high polarity, hydrophilicity and instability of the ozonation byproducts. An analytical method employing *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) derivatization and gas chromatography with electron capture detection (GC/ECD) has been reported for low molecular mass aldehydes in drinking waters.[11–13] In another development, Xiong *et al.*[14] used a method employing PFBHA and diazomethane derivatization to quantify glyoxylic acid in solutions of ozonated fulvic acid.

The objective of this study was to develop a gas chromatographic mass spectrometric (GC/MS) method to identify major keto acids in ozonated drinking water and fulvic acid samples.

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EXPERIMENTAL

Ozonated waters

Solutions of aquatic fulvic acid were used as model drinking waters. These were prepared at 4 mg l^{-1} dissolved organic carbon (DOC) from a concentrated fulvic acid aqueous stock solution which was isolated from Thousand Acre reservoir (Athol, MA, USA). These solutions were buffered at pH 7 with 10 mM total phosphates. They were then ozonated in batch by addition of a concentrated ozone stock solution. Ozone was applied at a dose ratio of 1 mg O_3 per mg DOC. Fulvic acid samples were allowed to react with ozone in the dark for 3 h before derivatization.

Sample derivatization

A derivatization method based on that reported by Xiong *et al.*[14] was used. In a glass bottle, 20 ml of PFBHA (Aldrich Chemical, Milwaukee, WI, USA) aqueous solution (6 mg ml^{-1}) was added to a 1000 ml unquenched water sample. This was incubated at 45 °C for 1.75 h, after which the sample was cooled to room temperature. Following the addition of 40 ml of concentrated sulfuric acid, the sample was extracted with 2 × 30 ml of methyl *tert*-butyl ether (MTBE) for 5 min in a separatory funnel. The combined MTBE extracts were concentrated to 2 ml under a gentle flow of nitrogen, then 2 ml of diazomethane MTBE solution, prepared in accordance with the method of Fales *et al.*,[15] was added to the concentrated extract. The extract was replaced in a refrigerator at 4 °C for 15 min. After quenching the residual diazomethane with silica gel, the

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extract was concentrated again to about 100 µl under nitrogen and submitted to GC/MS analysis.

GC/MS analysis

GC/MS analysis was carried out on an HP-5988 quadrupole mass spectrometer (Hewlett-Packard, San Fernando, CA, USA). The split–splitless injector was operated in the splitless mode, and a 30 m × 0.32 mm i.d. (0.25 µm film thickness) PTE-5 capillary column (Supelco, Bellefonte, PA, USA) was used. The column oven was kept at 50 °C for 2 min, then ramped to 250 °C at a rate of 4 °C min^{-1} and held at 250 °C for 8 min. The injector temperature was 200 °C and the transfer line was kept at 280 °C. In the electron impact (EI) mode, the ion source temperature was 200 °C, the electron energy was 70 eV and the mass scan range was 30–400 u. In positive chemical ionization (PCI) mode, the ion source temperature was 100 °C, the electron energy was 240 eV and mass scan range was 100–400 u. Methane was used as the reagent gas for PCI. Data were acquired and stored for subsequent analysis using an HP 59970 MS ChemStation. Other GC/MS operating conditions were similar to those reported previously.[16]

RESULTS AND DISCUSSION

A number of peaks were found in the total ion chromatogram of the derivatized extract from the ozonated fulvic acid sample, as shown in Fig. 1. Four monoaldehydes, with a predominant fragment at m/z 181, were identified, as shown in Table 1. The double peaks indicated the existence of isomers of derivatized acetaldehyde. There is no isomer for derivatized formaldehyde. No double peaks were observed for isomers of the derivatized propanal and butanal. This might be due to inadequate separation of aldehydes under the operating conditions, which were optimized for keto acids. All of these aldehydes have been reported by other researchers.[11–13]

Three other peaks, with fragments at m/z 59 and 181, were found in the methylated, PFBHA-derivatized extract. Without methylation none of these three peaks was observed. The EI mass spectra of these three peaks

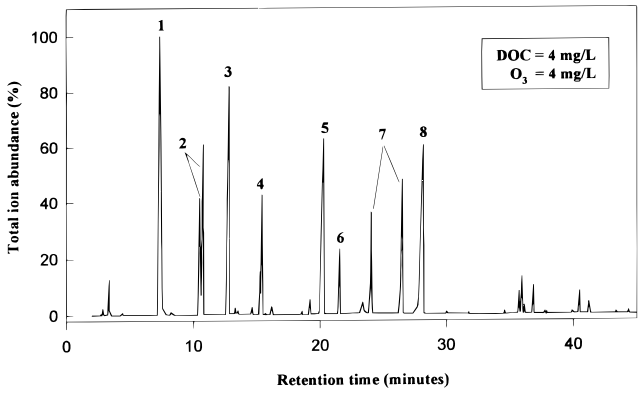

Figure 1. Total ion chromatogram of a derivatized ozonated fulvic acid sample.

in the double-derivatized extract of the ozonated fulvic acid solution are shown in Fig. 2. The *m/z* values of 59, 181 and 211 in these mass spectra indicate $[COOCH_3]^+$, $[C_6F_5CH_2]^+$ and $[C_6F_5CH_2ON]^+$ frag-

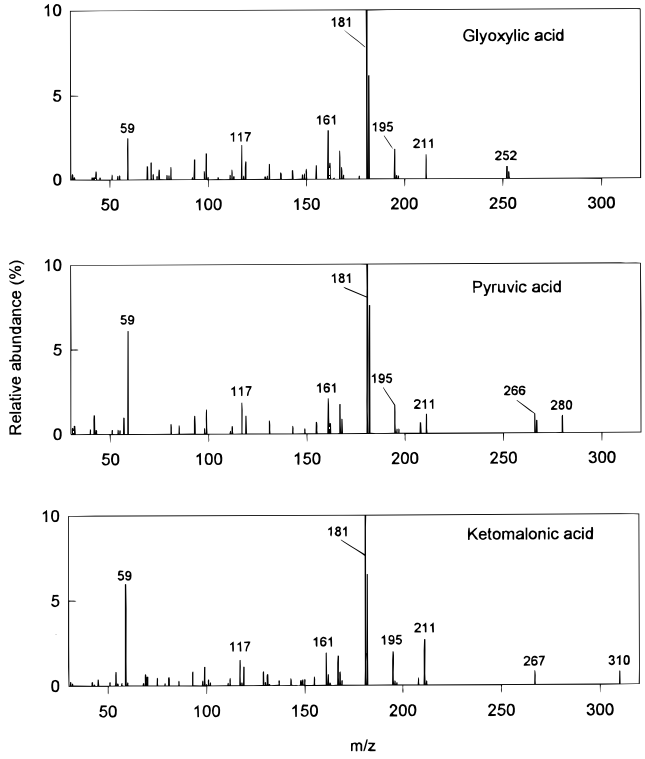

Figure 2. EI mass spectra of derivatized keto acids in ozonated fulvic acid solutions (base peaks 181 are 100%).

Table 1. Compounds identified in ozonated fulvic acid solutions

Peak No.	Proposed molecular mass	Proposed chemical structures: Derivatives	Ozonation byproducts
1	225	$C_6F_5—CH_2—O—N=CH_2$	H—CO—H
2	239	$C_6F_5—CH_2—O—N=CH—CH_3$	H—CO—CH_3
3	253	$C_6F_5—CH_2—O—N=CH—C_2H_5$	H—CO—C_2H_5
4	267	$C_6F_5—CH_2—O—N=CH—C_3H_7$	H—CO—C_3H_7
5	283	$C_6F_5—CH_2—O—N=CH—COOCH_3$	H—CO—COOH
6	297	$C_6F_5—CH_2—O—N=C(CH_3)—COOCH_3$	CH_3—CO—COOH
7	391	$C_6F_5—CH_2—O—N=CH—C_6F_5$	Analytical artifact
8	391	$C_6F_5—CH_2—O—N=CH—C_6F_5$	Analytical artifact
9	341	$C_6F_5—CH_2—O—N=C(COOCH_3)_2$	HOOC-CO-COOH

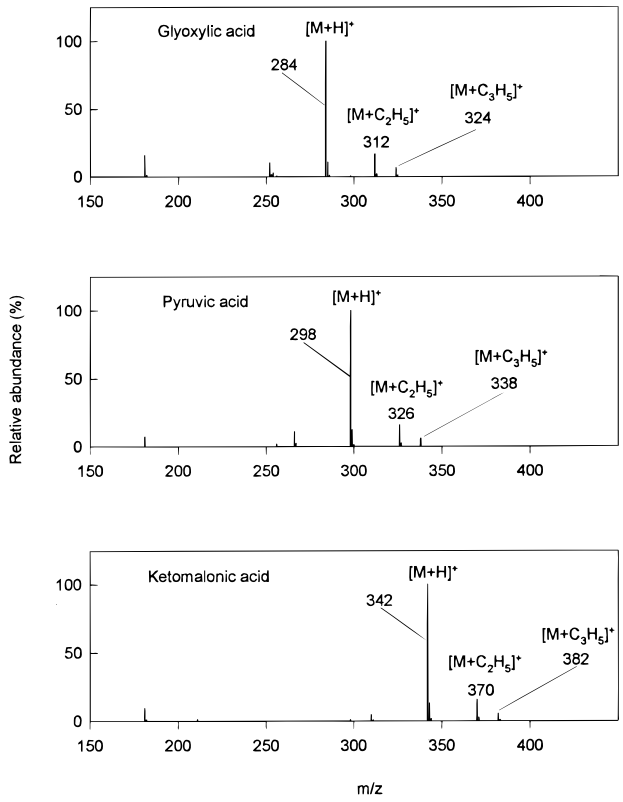

Figure 3. PCI mass spectra of derivatized keto acids in ozonated fulvic acid solutions.

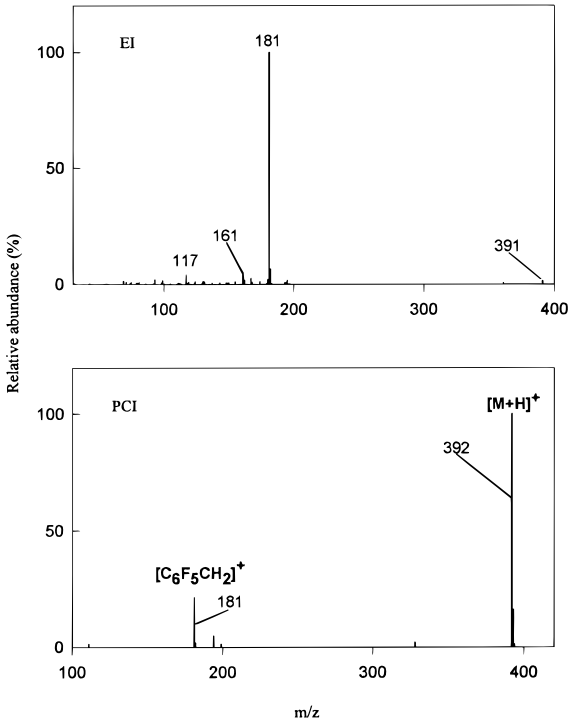

Figure 4. Mass spectra of an analytical artifact in ozonated fulvic acid solutions.

ments, respectively. For the fragment $[C_6F_5CH_2]^+$, the (A + 1) isotopic contributions for carbon for the three derivatives are 6.1, 7.6 and 6.5% of (A); respectively. These data match the theoretical isotopic contribution (7.7%) fairly well. These fragments suggest that the compounds contain carboxyl and carbonyl groups. Several other fragments higher than m/z 211 were also present at very low abundances. However, owing to the similarity of the EI mass spectra of these three peaks, it is difficult to assign definite structures to these mass spectra.

Table 2. Proposed fragment ion assignments for keto acid derivatives[a]

Proposed fragment ion	*m/z* of mass fragments (abundance, % of base peak)[b] Glyoxylic acid	Pyruvic acid	Ketomalonic acid
$[M-OH]^+$	266 (ND)	280 (1.0)	324 (ND)
$[M-NO]^+$	253 (0.4)	267 (0.7)	311 (ND)
$[M-OCH_3]^+$	252 (0.7)	266 (1.1)	310 (0.7)
$[C_6F_5CH_2ON]^+$	211 (1.4)	211 (1.1)	211 (2.6)
$[C_6F_5CH_2N]^+$	195 (1.7)	195 (1.6)	195 (1.9)
$[C_6F_5CH_2]^+$ [c]	182 (6.1)	182 (7.6)	182 (6.5)
$[C_6F_5CH_2]^+$	181 (100)	181 (100)	181 (100)
$[C_6F_5]^+$	167 (1.6)	167 (1.7)	167 (1.7)
$[C_6F_4CH]^+$	161 (2.9)	161 (2.0)	161 (1.8)
$[C_5F_3]^+$	117 (2.0)	117 (1.8)	117 (1.4)
$[COOCH_3]^+$	59 (2.4)	59 (6.1)	59 (6.0)

[a] Base peak is underlined; ND, not detected.
[b] The abundance is given in parentheses.
[c] (A + 1) isotopic contribution for carbon.

PCI was used to gain the molecular mass information on these peaks. The PCI mass spectra of a derivatized sample are shown in Fig. 3. The three ions in each PCI mass spectrum were assigned the structures $[M + H]^+$, $[M + C_2H_5]^+$ and $[M + C_3H_5]^+$. Therefore, from these PCI mass spectra, the molecular masses of these three compounds were determined to be 283, 297 and 341, respectively. By logical structural assignments for both the EI and PCI mass spectra (Table 2), these three peaks were identified as glyoxylic, pyruvic and ketomalonic acid derivatives. This result was also confirmed by matching the mass spectra of PFBHA–diazomethane-derivatized standards. These standards were prepared from the pure commercially available compounds, glyoxylic, pyruvic, and ketomalonic acid.

By using a similar derivatization procedure, Xiong *et al.*[14] identified glyoxylic acid in an ozonated fulvic acid solution. Mass spectra that resemble those of derivatives of pyruvic and ketomalonic acid were also presented by Xiong *et al.*, but, the authors were unable to assign definite structure to these mass spectra. Other studies suggest that the keto acids may be general ozonation products of a wide range of organic compounds. Using different derivatization techniques, glyoxylic acid has been found in ozonated solutions of acetic, maleic and oxalacetic acid and glyoxal.[17–19] Pyruvic acid was reported in an ozonated solution of 2-methyl-4-chlolophenoxyacetic acid.[20] Ketomalonic acid was reported in ozonated solutions of malonic acid, oxalacetic acid, tartronic acid, phenol, aniline, pheoxyacetic acid and *p*-cresol.[18, 20]

By comparison with an ozonated Super-Q water blank, two other major peaks were identified as analytical artifacts. These were apparently formed by the reaction between the residual ozone and PFBHA. Since

these two peaks were present in the unmethylated extract, this indicated that no carboxylic function groups were present in these compounds. By logical structural assignments of the EI and PCI mass spectra, as shown in Fig. 4, these two artifacts were identified as those shown in Table 1. The similar mass spectra and the same molecular masses indicated the existence of isomers. These two peaks were also reported as byproducts of residual chlorine and PFBHA.[12]

As reported here, these three keto acids have been found in ozonated drinking water at a level which is about 5–10 times higher than that of the aldehydes.[21–23] Since these keto acids are readily biodegradable, they can be removed through a biological filter after ozonation.[22] Owing to the insignificant level of keto acids in the biofilter effluent, they are unlikely to be of human health concern. The correlation between keto acid and assimilable organic carbon (AOC) has been reported previously.[22,23] The total keto acid concentration correlated well with AOC (NOX) in a variety of waters. This indicates that keto acid concentration is a potential chemical surrogate for AOC or biodegradable dissolved organic carbon (BDOC). Corroborating research was conducted by van der Kooij *et al.*,[24] who showed that glyoxylate and pyruvate clearly promoted the growth of strain NOX in tap water.

CONCLUSIONS

A GC/MS method combined with PFBHA–diazomethane double derivatization was developed to identify keto acids in ozonated waters. Three keto acids, glyoxylic, pyruvic and ketomalonic acid, were identified in ozonated fulvic acid solutions. Two analytical artifacts were also identified in the derivatized extract. Owing to the similarity of the EI mass spectra of keto acid derivatives, EI mass spectra together with PCI mass spectra are needed to identify these keto acids reliably.

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