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Detection, Identification, and Occurrence of Thiotetronic Acids in Drinking Water from Underground Sources by Electrospray Ionization-High Field Asymmetric Waveform Ion Mobility Spectrometry-Quadrupole Time-of-Flight-Mass Spectrometry

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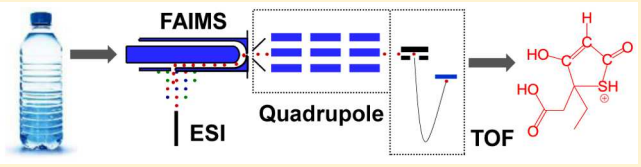
Supporting Information

ABSTRACT: This paper demonstrates that electrospray ionization (ESI) with differential ion mobility spectroscopy (FAIMS) and "soft" mass spectrometry (MS) provide unique analytical capabilities that led to the discovery of sulfur-containing polar congeners of thiotetronic acid (TA) in drinking water from underground sources in Canada and the United States. Polar TAs accumulate in underground aquifers and appear to be the most abundant class of organic compounds in bottled water but cannot be detected by conventional mass spectrometry methods. We show that normally stable TAs are converted into very reactive ions in ESI which have to be analyzed using special conditions in ESI-FAIMS-MS to avoid extensive dissociation and ion/molecule reactions. *De novo* identification of 10 TAs was accomplished by the comparative tandem mass spectrometry analysis of authentic TA derivatives from groundwater samples and synthetic TA analogues prepared for this study. We present highlights of gas phase ion chemistry of polar TAs to explain their unique properties and reactivity. TA derivatives were originally isolated from soil bacteria and are of interest in the pharmaceutical industry due to their potent activity against a broad spectrum of pathogenic bacteria and negligible toxicity to mammals. We suspect that TAs are natural disinfection agents protecting groundwater from bacterial contamination, but these compound undergo modifications or decompose during an ozonation water treatment.



As analytical techniques improve, many emerging organic micropollutants are being detected in groundwater.[1] Organic compounds of anthropogenic origin are of primary concern because they are generally responsible for the degradation of groundwater quality.[1,2] Liquid (LC) and gas chromatography (GC) with mass spectrometry (MS) are used in the analysis of preselected, target water contaminants of environmental importance, which are usually detected at low or negligible concentrations.[3] Groundwater also contains complex and chemically diverse dissolved natural organic matter (NOM) which appears to be released from the subsurface matrix and soil microorganisms.[4,5] Ultra high-resolution mass spectrometry (e.g., electrospray ionization-Fourier transform ion cyclotron resonance-mass spectrometry (ESI-FTICR-MS)) has been used to investigate the chemical composition of NOM.[5−7] This technique, however, is currently limited to the elemental analysis of individual components of NOM[5,6] and is not effective in their challenging identification on the molecular level. As a result, there are many unanswered question about the nature, fate, and biological properties of the natural organic components of groundwater.

Groundwater contains a characteristically high proportion of organic sulfur. On the basis of the elemental analysis, it is estimated that up to 20% of natural components of groundwater are currently unknown sulfur-containing compounds.[5] However, despite extensive water research in the last few decades, there is no literature report on molecular assessment of any major sulfur-contaminant. Another intriguing question relates to currently unknown bioactive compounds responsible for antibacterial activity of groundwater in bacterial bioassays such as Microtox and Eciox.[8] These tests are normally used to evaluate toxic responses from contaminated groundwater sources;[8,9] however, direct testing of groundwater from clean aquifers shows antibacterial activities that sometimes exceed toxic responses from chemically contaminated sites.[8] Assessments of groundwater by bacterial bioassays are complicated and compromised by a variable biological background activity that is not normally encountered in surface or tap water.[8]

We have shown that high field asymmetric waveform ion mobility spectrometry (FAIMS) facilitates selective detection of highly polar contaminants in tap water in a direct, quick, and convenient fashion without sample preparation, preconcentration, fractionation, chemical derivatization, or column separation. Consequently, FAIMS analysis provides high-quality spectral data for structural identification of new classes of previously unknown highly polar disinfection byproducts, even at low part-per-trillion concentrations.[10] FAIMS is a relatively new gas-phase atmospheric pressure separation technique[11]

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developed for use with MS,[12] and several FAIMS analyzers are commercially available.[13−16] FAIMS operates between ESI and MS as an ion filter[17] separating ions according to their differential ion mobility in alternating low and high electric fields generated in the gap between two closely spaced electrodes.[18] FAIMS separation is largely independent of mass/charge (m/z) and exploits ion size, shape, and polarizability, so it is orthogonal to MS and reversed-phase LC separation especially for highly polar species.[10]

In this paper, we will demonstrate the unique analytical capabilities of FAIMS combined with "soft" mass spectrometry in the analysis of extremely labile ions of sulfur-containing congeners of thiotetronic acid (TA). We will show that polar TAs appear to be the most abundant organic components in groundwater and bottled drinking water from underground sources. TAs have been of interest in the pharmaceutical industry since the first TA derivative thiolactomycin was isolated from soil bacteria and showed potent activity against a broad spectrum of pathogenic bacteria while having negligible toxicity to mammals.[19] The analytical challenges faced in the detection and identification of polar TA derivatives will be described to explain why these ubiquitous, abundant, and potentially bioactive compounds have never been detected in groundwater before.

EXPERIMENTAL SECTION

Ammonium acetate, formaldehyde, HPLC grade water, and methanol were purchased from Fisher Scientific (Nepean, Ontario). Groundwater samples were acquired at several locations in Ontario and bottled spring water was purchased from grocery stores in Guelph, ON; Edmonton, AB; and Chicago, IL. A TA synthetic analogue was synthesized and characterized by NMR according to a previously reported procedure.[20] Aside from a 10-fold dilution with methanol/water (9/1 v/v) containing 0.1 mM ammonium acetate, no sample clean up, preparation, or preservation was performed. Dilute water samples were infused into a nanospray source (Micromass, Manchester, U.K.) at a flow rate of 400 nL min^{-1} using the fluidics system of a nanoAcquity LC (Waters, Cambridge, MA) equipped with a 50 μL sample injection loop. Ionization was carried out with a spray voltage of +5000 V and −5000 V for positive and negative ion modes, respectively. The nanospray source was positioned 20° off axis of the orifice of a Selectra (Ionalytics, Ottawa) FAIMS device with a modified source interface described previously.[21] The FAIMS analyzer was operated at a dispersion voltage of 4000 V, outer electrode offset of ±150 V, and a curtain plate voltage of ±1000 V. N_2 and CO_2 gases (Linde, Guelph) were purified online by charcoal/molecular sieve filters, and a dry buffer/carrier gas (80% of N_2 and 20% of CO_2) was introduced into the FAIMS analyzer at a flow of 2 L min^{-1}. A Q-TOF micro mass spectrometer (Waters, U.K.) was used as a detector and ion transmission conditions (including the cone voltage of 14 V and the collision energy of 4 V) were optimized to minimize ion dissociation. Detection was carried out in either full scan (MS) mode while scanning compensation voltage (CV) or in tandem (MS/MS) mode at a constant CV. Grade 5 argon (Linde, Guelph) (H_2O < 3 ppm) was used as collision gas and collision energy (CE) was optimized for each experiment as shown in figure captions. The mass calibration was carried out using formaldehyde, as described recently for use with "soft" Q-TOF mass spectrometry.[22]

RESULTS AND DISCUSSION

Selectivity of ESI-FAIMS-MS in the Analysis of Groundwater. Figure 1 shows spectral data from the ESI-FAIMS-QTOF-MS analysis of raw, untreated groundwater from Ontario in the positive ionization mode. The compensation voltage (CV) spectrum (Figure 1A) was

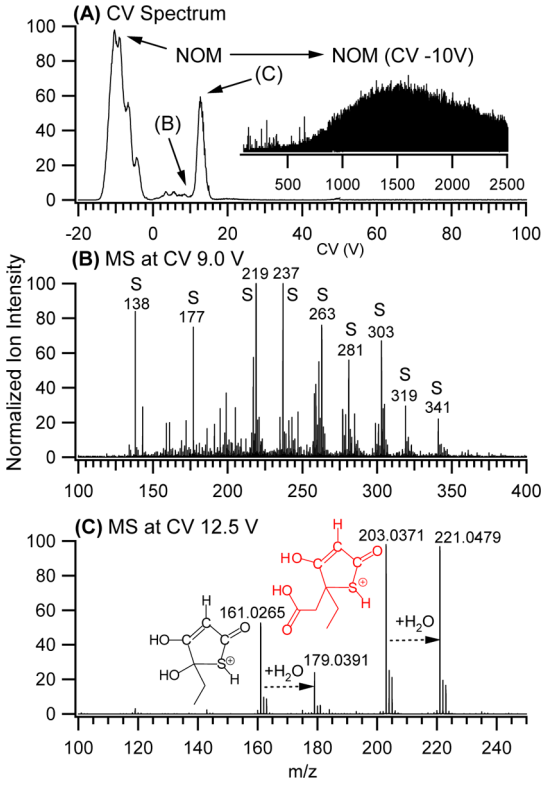

Figure 1. ESI-FAIMS-QTOF-MS analysis of raw groundwater from Ontario: (A) CV spectrum, MS spectrum of NOM at CV = −10 V in the inset; (B) mass spectrum at CV = 9 V; (C) mass spectrum at CV = 12.5 V. MS detection at SCV = 14 V and CE = 4 V using 20% of CO_2 in N_2 carrier gas. Sulfur-containing ions marked by "S".

acquired by scanning the CV from −20 to 100 V and detecting ions separated by FAIMS in full scan MS. High molecular weight components of dissolved NOM are transmitted in FAIMS in the CV range from −2 to −16 V. The mass spectrum (inset in Figure 1A) shows these ions, which have been detected together with smaller dissociation products at CV = −10 V. All mass spectra in the negative CV range demonstrate the enormous complexity of dissolved NOM in groundwater. FAIMS can separate these abundant spectral interferences from all other ions detected at the positive CV, yielding high quality spectral data. The MS spectrum at CV = 9 V (Figure 1B) contains a number of sulfur-containing organic compounds that could not be distinguished from the background of the groundwater matrix without the FAIMS separation. The most abundant sulfur-containing species, transmitted at CV = 12.5 V (m/z 203 and 161 in the MS spectrum in Figure 1C), have repeatedly been detected in groundwater samples from our local aquifers. The high selectivity of ESI-FAIMS-QTOF-MS was important for elemental and structural identification of these compounds. However, the detection of sulfur-containing contaminants is very challenging and requires unconventional experimental conditions that combine "soft" mass spectrometry detection and a high content of CO_2 in the FAIMS carrier gas.

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Detection of Labile Ions Using ESI-FAIMS and "Soft" Mass Spectrometry. Figure 2 shows spectral data from the ESI-FAIMS-QTOF-MS analysis of raw groundwater from Ontario using "soft" mass spectrometry and various CO_2/N_2 compositions of the FAIMS carrier gas. The diagram of ESI-FAIMS-QTOF-MS points to the most important ion optics parameters of QTOF-MS (the sample cone voltage at 14 V and collision energy at 4 V) that were optimized (see Supporting Information section S1) to minimize the ion kinetic energy and an extent of CID at the FAIMS-MS interface inlet and in the collision cell. These settings are ~3 times lower than those normally used in the analysis of environmental contaminants. At such conditions, even labile ions should be transmitted to the TOF detector without extensive fragmentation. However, the detection of sulfur-containing compounds (e.g., m/z 203 in Figure 2A) is observed only when the carrier gas contains a high proportion (20%) of CO_2. Addition of 10% CO_2 to the gas (Figure 2B) provides the detection of the same m/z 203 ion at much lower spectral intensity together with its more intense fragment ions at m/z 161, 143, 119, and 101. When pure N_2 is used in FAIMS, the m/z 203 ion is not detected (Figure 2C) and only fragment ions are observed at a very low spectral intensity. A similar trend is observed for slightly less labile ions of sulfur-containing species (m/z 161 and 175) which appear in the spectrum when CO_2 is used. Furthermore, a higher content of CO_2 decreases the differential mobility, shifting ion transmission CVs to less positive values (e.g., m/z 203; CV = 12.5 V in Figure 2A and CV = 14.5 V in Figure 2B).

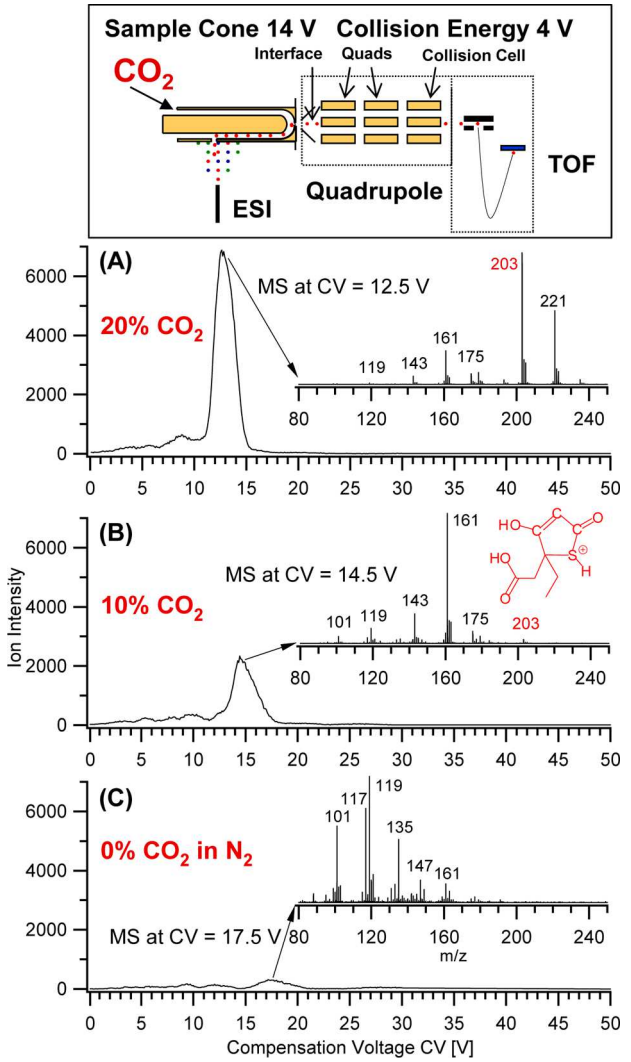

Figure 2. ESI-FAIMS-QTOF-MS analysis of raw groundwater from Ontario using "soft" mass spectrometry and CO_2 as a makeup gas in FAIMS. Schematic diagram of the ESI-FAIMS-QTOF-MS instrument is shown on the top pane. (A) MS spectrum at CV = 12.5 V using 20% of CO_2 in N_2 carrier/buffer gas in FAIMS, (B) MS spectrum at CV = 14.5 V using 10% of CO_2 in N_2 carrier/buffer gas in FAIMS, (C) MS spectrum at CV = 17.5 V using pure N_2 carrier/buffer gas in FAIMS. MS spectra in insets are shown on the scale representing normalized ion intensity.

The presence of carbon dioxide in FAIMS was shown to be critical for the detection of labile ions of trihalogenated acetic acids that could not be otherwise detected by ESI-MS.[23] Dramatically improved separation and detection capabilities were observed for these disinfection byproducts with 3−8% of CO_2 in the N_2 carrier gas.[23] However, much higher CO_2 content is required for detecting labile ions of sulfur-containing compounds. CO_2 is seldom used in mass spectrometry, but it is known as a "soft" collision gas that induces less extensive fragmentation than N_2 because inelastic collisions of CO_2 with an ion lead to vibrational excitation of CO_2 molecules.[24] Previous work suggests that CO_2 forms clusters with ions, and some of the energy imparted to a labile ion upon CID will be dissipated by breaking these clusters.[16] Consequently, increased content of CO_2 in the carrier gas suppresses CID and protects fragile ions from decomposition at several stages of the ESI-FAIMS-QTOF-MS analysis, including ESI (CO_2/N_2 is used as a curtain gas), FAIMS, and ion sampling, the MS inlet interface[25] where ion fragmentation preferably occurs.

Similarly favorable ion transmission conditions can be obtained when CO_2 is a part of the curtain gas in ESI without FAIMS. This "soft" mass spectrometry technique, however, is not capable of detecting abundant sulfur-containing water contaminants because they are not only very labile but also highly reactive electrophiles. In ESI-FAIMS-QTOF-MS, ion transmission into MS occurs in the pure carrier gas because other sample components are filtered out by FAIMS. As a result, the introduction of reactive ions at the MS interface can be carried out in a "clean" environment at "soft" sampling conditions. In ESI-QTOF-MS, reactive ions are introduced to the MS sampling interface together with solvents, salts, and ESI products from the complex water matrix. At such ion sampling conditions, reactive ions of sulfur-containing species we observe in ESI-FAIMS-QTOF are prone not only to dissociation but also to a wide range of ion/molecule reactions with nucleophiles such as water vapor. Groundwater samples from our study were subjected to analysis by several LC−ESI-MS, ESI-MS, or GC/MS systems using "soft" MS conditions on a variety of mass analyzers in several laboratories. These mass spectrometry techniques are not designed to deal with very labile and extremely reactive ions and were not capable of detecting any sulfur-containing compounds in groundwater.

Structural Identification of TAs. Structural elucidation of groundwater contaminants was carried out using a *de novo* identification approach[10,21] in which the manual interpretation of tandem mass spectrometry data for over 100 sulfur-containing compounds from groundwater allowed us to understand gas phase ion chemistry and establish structures of TA congeners. It is important to mention that there are no commercially available standards, reference spectra in databases, information in the literature, or established synthetic methods

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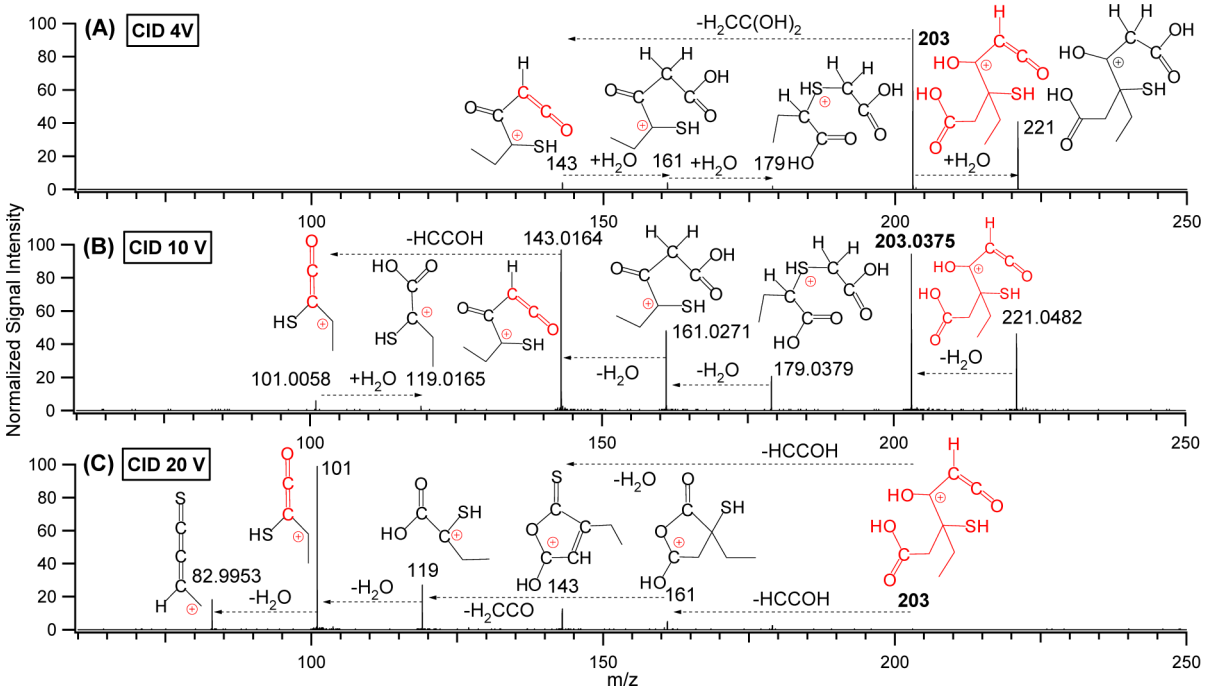
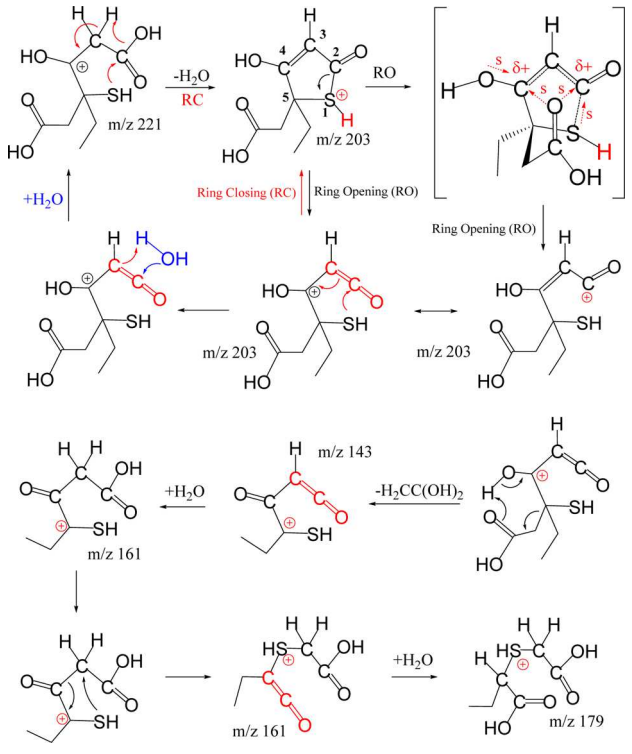


Figure 3. MS/MS spectra of the m/z 203 precursor ion of the TA detected in groundwater in Ontario at CV = 12.5 V and a collision energy of 4 V (A), 10 V (B), and 20 V (C).

for these new compounds. Molecular assessment of new TAs was supported by tandem mass spectrometry analysis of similar synthetic TA analogues prepared for our study to confirm ion chemistry of polar TA congeners, as described below. Figure 3 shows MS/MS spectra from ESI-FAIMS-MS/MS analysis of the major sulfur containing compound (m/z 203) which was detected in groundwater at CV = 12.5 V. The m/z 203 precursor generates several products at m/z 221, 179, 161, 143, 119, 101, and 83, and each product ion was additionally investigated in the pseudo MS^3 mode by inducing the source fragmentation of the precursor ion separated by FAIMS at CV = 12.5 V. Turning a QTOF with MS/MS capability into a pseudo MS^3 instrument with MS/MS/MS capability would not be possible without FAIMS due to the abundant chemical background from groundwater and ESI.[26]

At minimal ion activation conditions in CID (CE = 4 V in Figure 3A), the m/z 203 ion forms the water adduct at m/z 221. As shown in Scheme 1, this ion/H_2O reaction results from TA ring cleavage and involves an extremely reactive ketene group with an electron deficiency at the positively polarized middle carbon atom. Thiolactone ring opening and H_2O addition are reversible because the m/z 221 water adduct (pseudo MS^3 at CE = 4 V) showed water loss with the reformation of the TA ring. Analogous reactions were observed in the CID of protonated guanine where H_2O addition to a ketene moiety was attributed to reversible opening of the pyrimidine ring.[27,28] The heterolytic thiolactone bond cleavage proceeds through a transition state intermediate ion (Scheme 1) in which the positive charge density at C-2 increases progressively until the bond is broken. Stabilization of the intermediate arises from delocalization of the charge from the C-2 site as a result of interactions with the HS moiety and resonance through the adjacent (−C═C−OH) bond system. Further delocalization of the positive charge involves through-space interactions of the carbonyl oxygen of the CH_2COOH group. As a result of high stabilization of the charged

Scheme 1. Dissociation and Ion/H_2O Reactions of the m/z 203 Protonated TA (at the CE = 4 V) Detected in Groundwater at CV = 12.5 V[a]



[a]"S" indicates stabilization from charge delocalization.

intermediate, low activation energy is required for the cleavage of the thiolactone bond and TA ring opening for the m/z 203 occurs at extremely low collision energy (CE < 4 V). This ion/H_2O reaction could be attributed to a trace amount of water

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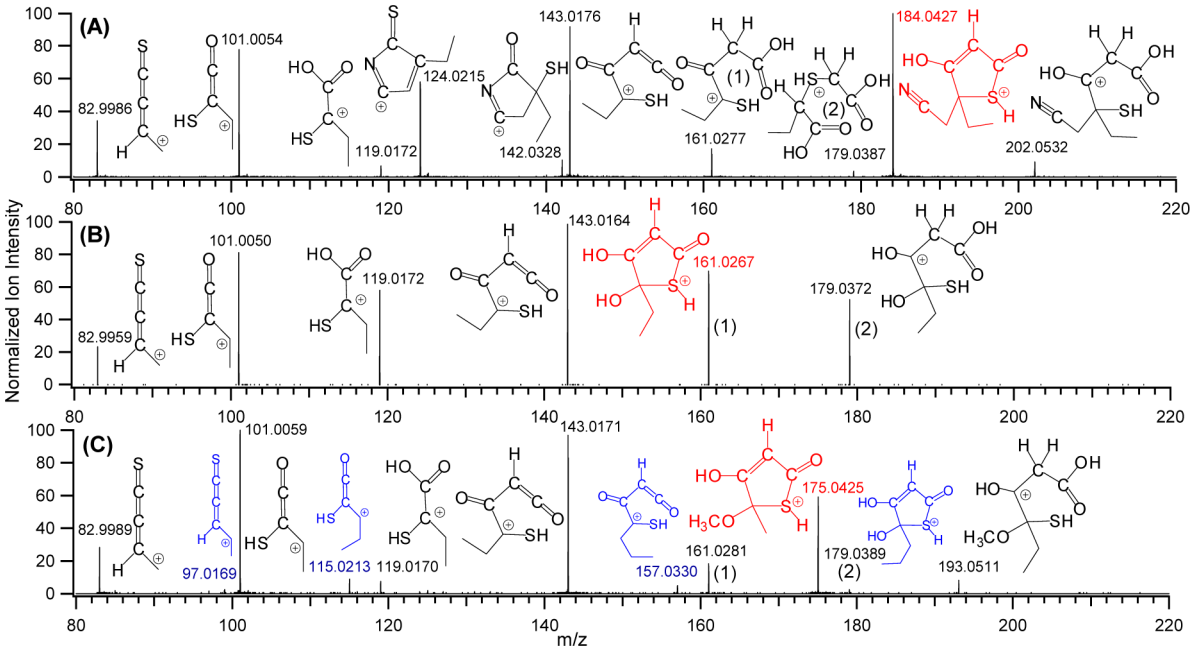

Figure 4. MS/MS spectra of precursor ions of major TAs detected in groundwater in Ontario at CV = 12.5 V. (A) MS/MS spectrum of the m/z 184 precursor ion, (B) MS/MS spectrum of the m/z 161 precursor ion, (C) MS/MS spectrum of the m/z 175 precursor ion.

vapor that is introduced into the collision cell with the argon collision gas. TAs are usually detected together with corresponding water adducts (Figure 1C), which helps in recognizing these compounds in ESI-FAIMS-MS spectra.

Initial fragmentation of the m/z 203 ion, observed also at a CE of 4 V, involves $CH_2{=}C(OH)_2$ elimination that leads to the m/z 143 dissociation product and two water adducts at m/z 161 and 179. As shown in Scheme 1, the detachment of the polar C-5 group from the m/z 203 precursor proceeds through the intermediate ion in which the positive charge at C-5 is stabilized by an adjacent SH group and the inductive effect of an ethyl group. The m/z 143 ketene fragment ion reacts with H_2O to produce the m/z 161 adduct which can accommodate, after rearrangement, another H_2O molecule to form another water adduct at m/z 179. Sequential dissociation of the m/z 143 ion (CE 10 V in Figure 3B) results in formation of the m/z 101 ketene ion (−HOCCH) and its m/z 119 water adduct. Further dissociation of the m/z 101 product (CE = 20 V in Figure 3C) generates the final dissociation product at m/z 83. Dissociation and ion/molecule reactions (observed at the CE = 4 V in Figure 3A and described in Scheme 1) allowed us to determine the TA ring structure and identify polar substituting groups present at the C-5 position. Additional reactions of the m/z 143 and alternative reactions of the m/z 203 precursor ion are described and discussed in Supporting Information (Scheme S2B,C).

We have identified 10 TA congeners (see Table S3 in the Supporting Information) which exhibit the same characteristic dissociation and ion/molecule reactions presented in Scheme 1. Figure 4 shows MS/MS spectra of precursor ions of other TAs which have been detected in groundwater samples at CV = 12.5 V. Each spectrum combines MS/MS data from CID at CEs of 4, 10, and 20 V to accommodate all product ions in one spectrum. Each precursor ion (m/z 184 in Figure 4A, m/z 161 in Figure 4B, and m/z 175 in Figure 4C) reacts with H_2O to form the corresponding water adduct at m/z 202, 179, and 193, respectively. Each initial neutral loss ($CH_2{=}C{=}NH$ from m/z 184 in Figure 4A, H_2O from m/z 161 in Figure 4B, and CH_3OH from m/z 175 in Figure 4C) represents the elimination of a polar C-5 group and formation of the m/z 143 ketene ion (Figure 4A−C) which is associated with water adducts at m/z 161 and m/z 179 (Figure 4A−C). All product ions discussed come from the reactions presented in Scheme 1. The dissociation of the m/z 143 ion generates product ions at m/z 101, 119, and 83 (Figure 4A−C). In addition, the m/z 184 precursor (Figure 4A) with a polar CH_2CN substituting group at C-5 generates m/z 142 (− HOCCH) and 124 (− HOCCH, − H_2O) products from dissociation pathways in Scheme S2C. The MS/MS spectrum of the m/z 175 precursor (Figure 4C) also contains low intensity fragment ions at m/z 157 (− H_2O), 115 (− H_2O, − HOCCH), and 97 (− H_2O, − HOCCH, − H_2O) which can be attributed to the TA with OH and C_3H_7 groups at the C-5 position. This minor TA isomer exhibits only major dissociation products from fragmentation pathways in Scheme 1 and Scheme S2B and less intense ion/H_2O reaction products are not detected in the spectrum. After establishing general dissociation patterns, it is not difficult to assign structures to TAs, including TA isomers that are transmitted in FAIMS at the same CV.

Our gas phase chemistry studies showed that TAs exhibit mechanistically similar ion dissociation and ion/H_2O reactions in CID, which makes their structural elucidation possible. TAs show no signs of degradation in groundwater samples collected over 3 years, but ESI converts normally stable TAs into very labile ions exhibiting the extensive dissociation at low activation conditions in CID. In addition, TA ions and several fragment ions with ketene groups are extremely potent electrophiles which react even with trace amounts of H_2O in the collision cell of QTOF-MS. Ketene chemistry presents more problems during the detection of TAs by conventional ESI-MS techniques because TA ions are involved in a wide range of ion/molecule reactions at the MS sampling interface with nucleophiles from solvent vapor, salts, buffers, and other species from a water sample. The FAIMS technique can address not

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only the fragile but also reactive nature of TA ions by providing the clean environment and gentle transmission conditions during the introduction of ions into the mass spectrometer.

Analysis of TAs as Negative Ions. Groundwater TAs can also be analyzed as deprotonated molecules using negative n-ESI-FAIMS-QTOF-MS with "soft" mass spectrometry as shown in Figure 5. The CV spectrum (Figure 5A) includes

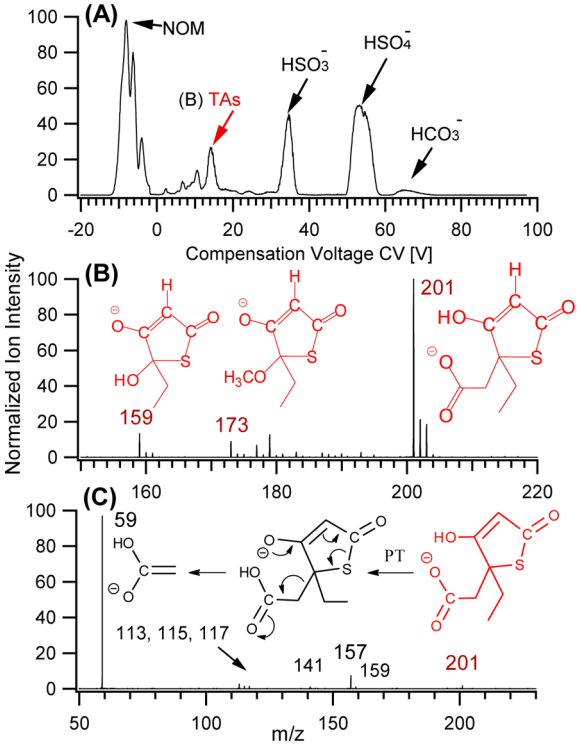

Figure 5. ESI-FAIMS-QTOF-MS analysis of a groundwater sample from Ontario with negative ESI: (A) CV spectrum, (B) MS spectrum at CV = 14 V, (C) MS/MS spectrum of the m/z 201 precursor ion at CV = 14 V.

negatively charged ions of dissolved NOM in the CV range from −2.0 to −12.0 V and several inorganic anions which are separated at larger CV values. Deprotonated TAs (m/z = 201, 173, and 159) were found in the MS spectrum (Figure 5B) at CV = 14 V. Elemental composition and results from tandem mass spectrometry confirmed the detection of the same TAs observed in the positive mode at the CV = 12.5 V. Figure 5C shows the CID MS/MS spectrum of the m/z 201 precursor ion that dissociates extensively at low collision energies (from 4 to 20 V) to produce one major fragment ion at m/z 59. Other dissociation products at m/z = 159, 157, 141, 117, 115, and 113 were detected at much lower spectral intensities. To obtain high-quality MS/MS spectra for the precursor ion (Figure 5C) and MS^3 spectra for fragment ions, we used extended data acquisition times (up to 1 h). This approach allowed us to establish dissociation pathways of the m/z 201 and structures of fragment ions, presented in the Supporting Information (Scheme S4A−C). Gas phase chemistry of negative TA ions is less informative for structural elucidation of new congeners but very useful for the confirmation of structural assignments from the positive mode. It is important to note that while extended data acquisition times were important for establishing the reactivity of and identifying new TAs, ESI-FAIMS-MS analysis of TAs in drinking water once they have been identified is quite rapid. Typical scan times for the screening of water samples ranged from 2−10 min depending on requirement of screening low abundance TAs. Deprotonated TAs were detected in ESI-FAIMS-QTOF-MS together with inorganic components of groundwater such as HSO_4^- (at the CV = 54 V in Figure 5A). HSO_4^- is the ionization product of sulfate that was present in the analyzed groundwater sample at the concentration of 60 mg L^{-1}. The analytical signal (peak area around the CV of 54 V) for HSO_4^- is approximately 10 times larger than the peak area for major TAs around the CV of 14 V. Assuming similar ionization efficiencies for sulfate and TAs, one can roughly estimate that the most abundant TAs are present in Ontario's groundwater at a low mg L^{-1} concentration level but more accurate concentration (7.3 mg L^{-1}) was obtained from the internal standard quantification method (see Supporting Information section S5).

Occurrence of TAs. Underground aquifers are sources of bottled drinking water widely distributed in Canada and the United States. Figure 6 illustrates the positive ESI-FAIMS-QTOF-MS analysis of bottled drinking water supplied by the same major food company from several distant underground

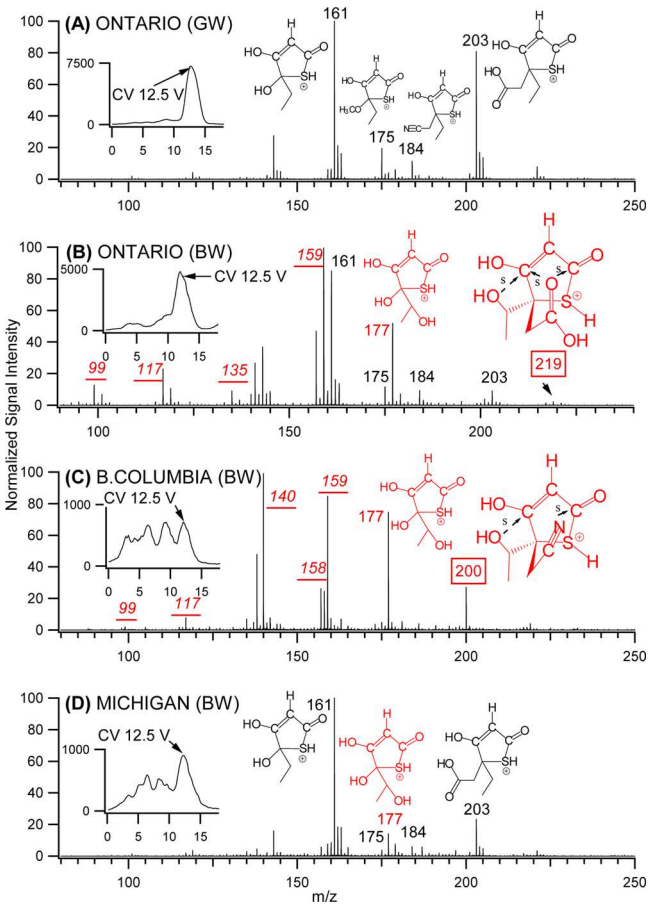

Figure 6. MS spectra from the ESI-FAIMS-QTOF-MS analysis of bottled drinking water from underground sources (CV = 12.5 V). CV spectra are shown in the insets: (A) raw water from an underground aquifer in Ontario, (B) bottled drinking water from the same underground aquifer in Ontario after ozone purification treatment; m/z indicate fragment ions of the m/z 219 ion, (C) bottled drinking water from an underground aquifer in British Columbia after ozone purification treatment; m/z indicate fragment ions of the m/z 200 ion, (D) bottled drinking water from an underground aquifer in Michigan after reverse osmosis purification treatment.

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sources. Raw groundwater from our local artesian aquifer (Figure 6A) contains TAs (m/z 203, 184, 175, and 161) detected at the CV = 12.5 V in the CV spectrum shown in the inset in Figure 6A. Groundwater from the same source, after ozone purification treatment, is used to produce bottled spring water (Figure 6B) for Eastern Canada. In addition to residual TAs (m/z 203, 184, 175, and 161), this spring water also contains ozonation products of TAs (m/z 203 and 161) representing more polar hydroxylated TAs (m/z 219 and 177) which are more difficult to detect as intact molecular ions. In particular, the highly polar TA at m/z 219 (barely detected at CV = 12.5 V in Figure 6B) has two C-5 polar groups and dissociates extensively to generate intense product ions at m/z 159, 135, 117, and 99. Formation of these ions occurs by the same dissociation and ion/H_2O reactions presented in Scheme 1 and Scheme S2B,C for the less polar TA at m/z 203. However, the additional OH group at the α-carbon of the ethyl group provides additional stabilization for transition state intermediates (such as the intermediate ion in Figure 6B) and facilitates the initial cleavage of the TA ring as well as all other sequential dissociation reactions of this very labile molecular ion. Bottled spring water from an underground aquifer in British Columbia (Figure 6C) is also ozone-treated and distributed in Western Canada. It contains polar TA derivatives (m/z 177 and 200) from the ozone treatment of residual TAs (m/z 161 and 184). The labile m/z 200 molecular ion with two charge-stabilizing C-5 polar groups dissociates extensively according to general reactions pathways of TAs (Scheme 1, Scheme S2B,C and Figure 4A), but its fragments (m/z 159, 158, 140, 117, and 99) are among the most intense ions in the MS spectrum (Figure 6C) due to the presence of an OH group at the α-carbon of the ethyl group. Bottled spring water (Figure 6D) from an underground source in Michigan State is purified by reverse osmosis and contains the same TAs as groundwater from Ontario (Figure 6A). TA derivatives were not detected in Ontario's groundwater subjected to chlorine disinfection and detected only at a trace level after UV treatment.

The analysis of bottled drinking water demonstrates that common TAs are present in distant underground sources, although a water treatment can alter their abundance and generate more polar TA disinfection byproducts. Occurrence of TAs in groundwater is not restricted to groundwater in Ontario. TA derivatives appear to be the most abundant organic compounds in bottled drinking water that is consumed by millions of individuals in Canada and the United States.

Chemical and Biological Properties of TAs. TA congeners from our study are structurally related to a TA congener thiolactomycin (TLM), which was originally isolated from soil bacteria.[29] TLM exhibits moderate *in vitro* and potent *in vivo* activity against many pathogens such as Gram-positive and Gram-negative bacteria[30] including *Mycobacterium tuberculosis*[31] and malaria parasites.[32] TLM inhibits the type II cell wall fatty acid synthesis (FAS II) in bacteria and plants,[33] which explains the antibacterial and antiparasitical properties of TLM. Because TLM is not toxic in mammals,[30] over 100 synthetic, mainly nonpolar TLM congeners have been investigated to establish structure activity relationships (SAR) and molecular mechanisms of action of drug candidates with the common TA ring system.[19] According to published studies, binding at the active site of FAS II enzymes (Figure S6-1) can explain *in vitro* inhibitory activities of TA derivatives like TLM.[34,35] Biological activity of TAs depends on substitution patterns and tautomerism (see the Supporting Information Scheme S6). Groundwater TAs should be potent FAS II enzyme inhibitors and new synthetic methods are being investigated to synthesize these unique compounds for ultimate identification, quantification, and evaluation of biological activity. Our ESI-FAIMS-MS method can be also applied in pharmaceutical research where the lack of an appropriate analytical technique for studying metabolism of TAs has hindered the development of new antibiotics.

CONCLUSIONS

We have demonstrated the unique analytical capabilities of ESI-FAIMS-QTOF-MS for the detection and identification of the most abundant natural organic components in groundwater. We suspect that TAs are produced by soil bacteria and especially polar TAs can be transported through soil and accumulate in underground aquifers. Occurrence of TAs in bottled drinking water from distant underground sources in Canada and the United States indicates that groundwater is poorly characterized with respect to major natural components. This work describes a method that can be used to address limitations in the detection of labile compounds using conventional mass spectrometry techniques. Polar TA derivatives represent only one class of compounds among many other labile water contaminants that could be identified using ESI-FAIMS-MS. We have recently implemented this method for detecting labile ions in the analysis of highly polar sulfur-containing metabolites, carbohydrates, and synthetic organic compounds as well.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b02372.

Additional information on detection, gas phase ion chemistry, occurrence, quantification, and chemical/biological properties of thiotetronic acids (PDF)

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Notes

The authors declare no competing financial interest.

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